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The kinetics of the title system has been investigated by the Lewis cell technique for 1-day ageing of the aqueous phase and compared with those obtained from the single drop technique. The mass transfer flux equations for Zr(IV) have been derived for three aqueous acidities of 0.10, 1 and 5 mol dm\(^{-3}\) HCl, respectively, as:

\[
J = 10^{-5.53 \pm 0.04} (1+0.00038[Zr(IV)]^{-1})^{-1} [H^+] [H_2A_2^0] (o) (1+0.70[Cl^-])
\]

\[
J = 10^{-5.80 \pm 0.02} (1+0.004[Zr(IV)]^{-1})^{-1} [H_2A_2^0] (o) [Cl^-]
\]

\[
J = 10^{-6.58 \pm 0.03} (1+0.0038[Zr(IV)]^{-1})^{-1} [H^+] [H_2A_2^0] (o) [Cl^-].
\]

The values of \(E_a\) (kJ mol\(^{-1}\)) in kinetic and diffusion regimes are 92 \& 14.7 and 84 \& 15 for 1 and 5 mol dm\(^{-3}\) HCl systems, respectively. For 0.10 mol dm\(^{-3}\) HCl system, \(E_a\) value cannot be measured for kinetic regime but its value is 15 kJ mol\(^{-1}\) in diffusion regime. At intermediate controlled regime (Zr(IV) \(\approx 3\) mmol dm\(^{-3}\)), \(E_a\) value varies from 11, 12 and 13 kJ mol\(^{-1}\) to 42, 105 and 108 kJ mol\(^{-1}\) respectively, for 0.10, 1 and 5 mol dm\(^{-3}\) HCl systems on varying the temperature from 318 K to 288 K.

On the basis of these data, the mechanisms of extraction in different conditions have been suggested.

**Key words:** Extraction kinetics, Zr(IV), D2EHPA, Lewis cell, Kerosene HCl interface.

---

**Introduction**

The extraction equilibria of the Zr(IV)-Cl-D2EHPA-kerosene system has been reported (Biswas and Hayat 2002a). Extraction equilibria have been found to be complicated by the slow change in the composition of the extractable aqueous Zr(IV) species on ageing and variation of the aqueous phase acidity. It is found that the equilibrium data for 1-day ageing can be justified well if the existences of \([\text{Zr}_8(\text{OH})_{20}(\text{H}_2\text{O})_{24}\text{Cl}_{12}]\) in 0.10 mol dm\(^{-3}\) HCl medium (Singhal et al 1996), \([\text{Zr}_4(\text{OH})_8(\text{H}_2\text{O})_{16}\text{Cl}_4]^{2+}\) in 1 mol dm\(^{-3}\) HCl medium (Singhal et al 1996) and \([\text{Zr}_8(\text{OH})_8(\text{H}_2\text{O})Cl]^+\) in 5 mol dm\(^{-3}\) HCl medium (Hannane et al 1990) are considered to take part in extraction equilibration reactions. On ageing for 30 days, the above species take up 1, 2 and 3 chloride ions, respectively. For 1-day ageing, the extraction equilibrium constants have been estimated to be \(10^{-5.0}\), \(10^{-4.1}\) and \(10^{-3.4}\) for 0.10, 1 and 5 mol dm\(^{-3}\) HCl systems, respectively.

In another paper (Biswas and Hayat 2002b), the kinetics of the title system of 1-day ageing has been measured by the single drop technique. The rate constants have been measured to be \(10^{-5.37}\), \(10^{-5.77}\) and \(10^{-6.62}\) for 0.1, 1 and 5 mol dm\(^{-3}\) HCl systems, respectively. According to Danesi and Chiarizia (1980), a change in the experimental technique and concentration condition may alter a kinetic regime to a diffusional regime or mixed control or vice versa. Now, it is established that not only the concentration term, but also the temperature condition alters the mechanism of extraction (Hughes and Biswas 1991 and 1993; Biswas and Begum 2000; Biswas and Mondal 2003). This paper discusses the kinetics of the Zr(IV) extraction from Cl medium of 1-day ageing by D2EHPA in kerosene by the Lewis cell technique to compare these results with those obtained from the single drop technique.

**Experimental**

**Reagents.** D2EHPA was procured from BDH (98% purity) and used as such. As a source of Zr(IV), octahydrated zirconyl chloride (M.C and Bell, 98%) was used. Kerosene was bought from the local market and redistilled to collect the fraction distilling over 220-260°C. It was mostly colorless and aliphatic in nature. All other chemicals were of reagent grade and used without further purification.

**Analytical.** The concentration of Zr(IV) in the aqueous phase was estimated by the EDTA-pyrocatechol violet method (Charlot 1964) at 590 nm using a WPA S104 spectrophotometer. For pH adjustment of the aqueous solution of Zr(IV) required in the above method (pH5.2), a Mettler Toledo 320 pH meter was used.
SYNTHESIS OF 5, 7-DIHYDROXY-6, 8-DI-C-PRENYL-4-O-PRENYL-FLAVANONE

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The prenylated flavanone (9) has been synthesized from phloroacetophenone (1). All the new products have been characterized by the spectral data and microanalysis.

Key words: Synthesis, Chalcone, Flavanone

Introduction

Flavones and their derivatives are naturally occurring and have a variety of biological properties, such as antibacterial (Mitscher et al 1993), antifungal (Conn 1981) and antitumour activity (Mizabuchi and Sato 1984). A large number of natural products including flavonoids are being reported in the literature every year and their structures need to be confirmed by synthesis. This paper reports the synthesis of 5,7-dihydroxy-6, 8-di-C-prenyl-4′-O-prenylflavanone (9) from phloroacetophenone (1), which may be used as a synthetic marker. Phloroacetophenone on treatment with methoxy-methyl chloride using K 2 CO 3 and acetone afforded 2-hydroxy-4, 6-di-(methoxymethoxy) acetophenone (2) (Hossain 1999), which on nuclear prenylation using well-cooled solution of KOH and prenyl bromide gave three products viz 2-hydroxy-4, 6-di-(methoxymethoxy)-3-C-prenylacetophenone (3), 2-hydroxy-4, 6-di-(methoxymethoxy)-5-C-prenylacetophenone (4) (Hossain and Islam 1993), and 2-hydroxy-4,6-di-(methoxymethoxy)-5, 6-di-C-prenylacetophenone (5) and several other minor products. Similarly O-prenylation of p-hydroxybenzaldehyde using K 2 CO 3 /acetone/prenyl bromide gave 4-O-prenylbenzaldehyde (6). Alkaline condensation of 2-hydroxy-4, 6-di{(methoxy-methoxy)-3, 5-di-C-prenylaceto-phenone (5) and 4-O-prenylbenzaldehyde (6) yielded 2′-hydroxy-4′, 6′-di-(methoxymethoxy)-3′, 5′-di-C-prenyl-4-O-prenylchalcone (7). Compound (7) on treatment with NaOAc/EtOH furnished 5,7-di-hydroxy-6, 8-di-C-prenyl-4′-O-prenylflavanone (8), which upon demethoxymethylation afforded 5,7-dihydroxy-6, 8-di-C-prenyl-4′-O-prenylflan-9-one (9).

Experimental

Melting points were determined using an electrothermal melting point apparatus (Gallenkamp) and are uncorrected. IR spectra were recorded (KBr discs) on a Pye-Unicam SP3-300 IR spectrophotometer (ν max in cm −1 ), 1H-NMR spectra on a Varian 300 MHz instrument in CDCl 3 with TMS as an internal standard (chemical shifts in δ, ppm) and UV spectra on Milton-Roy UV-visible spectrophotometer Ultrospeck in methanol (λ max in nm). TLC was performed using silica gel 60G. Mass spectra were recorded on Time of Flight (GC-MS TOF). Satisfactory elemental analyses were obtained for all the compounds and structures are in accord with the UV, IR and 1H-NMR data.

Methoxymethylation of phloroacetophenone (1). A mixture of phloroacetophenone (1), 10 g) in dry acetone (100 ml), methoxymethyl chloride (5.67 g) and anhydrous potassium carbonate (40 g) was refluxed for about 3 h. The progress of the reaction mixture was examined by TLC. On completion of the reaction acetone was distilled off and water was added and the mixture was then extracted with ether washed with water and dried over anhydrous Na 2 SO 4 . The organic layer was evaporated to dryness. The ether extract on silica gel column chromatography (mesh 60-120) using petrol (40-60 °), petrol-benzene (4:1), petrol-benzene (4:3) and increasing quantities of benzene as eluent gave the major compound (2) and several other minor compounds. Compound (2) was purified from column and by preparative TLC over silica gel GF 254 using benzene-petrol (25:1) as developing solvent. It was crystallized from petrol as colorless crystals (4.09g) R f 0.69 (benzene-petrol; 25:1); m.p 80-81 °C; IR: 3450, 2874, 1654, 1610, 1476, 1365, 1275, 1234, 1190, 1156, 1064, 1034, 996 984, 934, 886; 1H-NMR: 2.45 (s,1H,1-COCH 3 ), 3.45 (s,6H,-CH 2 OCH 3 x2), 5.55 (s,4H,-CH 2 O-CH 3 x2), 6.45 (s, 1H,H-3), 6.67 (s, 1H H-5), 12.76 (s, 1H, -OH).

Nuclear prenylation of 2-hydroxy-4, 6-di{(methoxy-methoxy) acetophenone (2). 2-Hydroxy-4,6-di-(methoxy-methyleneoxy) acetophenone (2, 1g) was added to a well cooled solution of KOH (2 g) in absolute methanol (30 ml)
**Effect of pH and Concentration on the Removal of Magnesium from Magnesium Chloride Solution by Bentonite**

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(Received July 10, 2002; accepted August 2, 2004)

By virtue of their well-known cation exchange capacities of clays, particularly bentonites are important minerals to be used as adsorbent of various undesirable ions drained out as industrial waste. One of such pollutant is magnesium containing waste. The removal of magnesium from magnesium containing solutions is found to be dependent both on pH of the solution and the concentration of magnesium ions present in the solution. Using magnesium chloride solution of 50 mg/lit and 100 mg/lit concentration and bentonite (North West Frontier Province), it was found that adsorption equilibrium established within 6 h.

**Key word:** Bentonite, Adsorption, Freundlich equation

**Introduction**

Industries such as medicine, toiletry, printing ink, refractories, rubber insulating material and some other chemical industries extensively use magnesium compounds in their manufacturing processes. In sewerages of these industries the concentration of the magnesium ions is generally above the permissible limit (50 ppm), which causes deleterious effects to human health. Clays have been used for the adsorption of organic molecules (Jhonston and Cardile 1987) inorganic metallic ions (Diez 1980) and dyestuff, in waste water. In the present study a Pakistani bentonite is used for the removal of magnesium ions from magnesium chloride solution. Bentonites are essentially hydrous aluminium silicates, with magnesium or iron, alkali or alkaline earth metals (Grim 1962). They are composed of small crystalline particles of one or more clay minerals. The structure of bentonite is composed of a single silica tetrahedral sheet and a single alumina octahedral sheet combined in a unit in such a way that the corners of silica tetrahedron and the layer of octahedral sheet form a common layer. All the corners of silica tetrahedrons point in the same direction and towards the centre of unit (Mitra et al. 1979). Cleavage between sheets leads to plate-like particles and leave these layers unsaturated.

The ion exchange ability of bentonite is probably due to, i) Broken bonds due to sub-division of the giant crystal. ii) Disordered structure containing ions of incorrect valence. Where, there are unsaturated bonds or electrical charges, their counter ions can be adsorbed. This effect would be dependent on particle size (Worral et al. 1958). The second cause of charged clay particles is the structural disorder due to isomorphism substitution of Al$^{3+}$ for Si$^{4+}$ and Mg$^{2+}$ for Al$^{3+}$, giving rise to inherent negative charges. This is independent of particle size (Whittaker 1939). In addition, some anions may be adsorbed by the replacement of exposed hydroxyl ions and because of the structural arrangement of some of the anions with tetrahedral units (Foster 1951). This absorption behaviour of bentonite was observed to be influenced by various factors such as contact time, solution concentration (Miyazaki 1996), temperature (Shakila et al. 1998) and pH (Compton et al. 1994).

The present work has been undertaken with a view to investigate the adsorption characteristics of bentonite for the adsorption of magnesium from magnesium chloride solution and the effect of concentration of magnesium, pH of the salt solution on the extent of adsorption of magnesium.

**Experimental**

In present studies, bentonite from Peshawar area was used. Snow’s method is used to determine the surface area (Iqbal 2001). The chemical analysis of the bentonite and other characteristics are given in Table 1. The method is described as below.

**Determination of surface area of bentionite.** Potassium iodide (KI) 71.3 g and 7.5 g of iodine (I$_2$) crystals were dissolved in a small quantity of distilled water and the volume made up to 125 ml. About 1 g of washed bentonite was taken in an iodine flask and 5 ml of iodine solution was added. It was allowed to stand for 2 h. Then 45 ml of distilled water was added and flask was gently spiralled for 1 min. It was kept for 1 h, 20 ml of the supernant solution was taken in titration flask and two drops of starch solution was added. A
Introduction

Road traffic noise is the most widespread source of noise in all countries and the most prevalent cause of annoyance and interference. Traffic noise surveys conducted in Karachi by Shaikh et al. (1987 and 1997) and Hyderabad by Shaikh and Shaikh (2000) shows that in (i) Karachi with the exception of a few occasional peaks, the levels of traffic noise levels vary in the range of 61 to 97 dB(A), with LA90, LA50 and LA10 values in the range of 70.1 - 78.4, 79.6 - 84.5 and 85.6 - 90.8 dB(A), respectively and (ii) Hyderabad in the range of 57.1 - 101.9 dB(A), with LA99, LA90, LA50, LA10 and LA1 values in the range of 60.4 - 73.3, 66.2 - 79.6, 75.2 - 82.8, 85.0 - 90.9 and 89.1 - 99.0 dB(A), respectively and LAeq12h values in the range of 81.2 - 86.9 dB(A). These levels are excessively high and much above the community annoyance limits recommended by the International Standards Organization (ISO) and some other individual countries. Roadside dwellers and traders are constantly exposed to such a high level noise for about more than 12 h a day.

The result of another survey (Ahmad 1992; Ahmad 1994) in Karachi, Lahore, Faisalabad, Hyderabad and Sukkur shows that the levels of traffic noise in these cities vary in the range of 72 - 95, 74 - 90, 70 - 92, 60 - 90 and 60 - 85 dB(A), respectively. However, the methodology used by these surveys, such as (i) most of the readings reportedly taken in dB (ii) distance of the meter from the nearest line of flow of vehicles (iii) time weighting (iv) fewer readings (v) average values generally based on minimum and maximum readings and (vi) incorrect range of values raises questions about the credibility of the results and inferences to made thereof (Shaikh and Shaikh 2000; Shaikh 2003). The Environmental Protection Department, Lahore, has reported traffic noise levels for six places in Lahore, in the range of 26 - 121 dB (not dB(A) (EPD 1996). For Village Bath, Lahore, traffic noise levels have been reported in the range of 26 - 50 dB (not dB(A), which is unimaginable and may have been occasioned by technical problem in the measuring equipment (e.g. battery voltage drop). EPA’s measurement of traffic noise levels with the handheld device inclined at about 45 degree was irregular and rendered the results unreliable. More standard measurement procedures were employed in the surveys carried out by Shaikh et al. (1987, 1997 and 2001).

Therefore, in order to have detailed assessment of prevailing road traffic noise in different areas and localities, traffic noise survey was conducted at 15 sites on busy roads with heavy traffic density in the residential and commercial areas of Lahore city. Due to the absence of proper regulatory laws to limit high-level traffic noise in Pakistan, the results are discussed with reference to the community annoyance criteria, recommended by ISO and some other individual countries. Some suggestions for limiting highlevel traffic noise are also discussed.

Materials and Methods

Measuring instruments and techniques. The measuring instrument consisted of a Sound Level Meter. The meter was regularly calibrated against an acoustic calibrator and checked before and after each series of measurements. During all the measurements, the meter was kept at 1.5 m above the ground level and at a distance of about 5 m from the edge of the nearest

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**Phytochemical Analysis and Laxative Activity of the Leaf Extracts of Euphorbia heterophylla Linn (Euphorbiaceae)**

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(Received July 26, 2003; accepted August 23, 2004)

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A hot aqueous decoction of the leaves of *Euphorbia heterophylla* Linn (Euphorbiaceae) 1.24 kg gave on cooling and defatting with dichloromethane, an aqueous solution which on successively extracting with n-butanol and ethylacetate gave 25.89g and 1.31g of residue, respectively on removal of solvent. The semi-solid extract from the ethylacetate fraction on hydrolysis with dilute tetraoxosulphate (VI) acid gave a yellow powder which on acetylation gave colourless needle clusters identified as quercetin tetracetate. The butanolic fraction had laxative action and contained saponins, phenols, terpenes and diterpenes identified as phorbols but no anthraquinones. The residual aqueous solution contained mainly sugars identified as xylose, maltose, galactose, lactose and lactulose, which are bulkforming laxatives. The purgative action was found to be a joint action of both the phorbols in the butanol fraction and the bulk forming laxative sugars in the residual aqueous fraction.

**Key words:** *Euphorbia heterophylla*, Laxative activity, Tetraoxosulphate (VI).

---

**Introduction**

Hot aqueous decoctions of *E. heterophylla* leaves have been used for ages to produce purgation in the southeastern part of Nigeria (Gill 1992). The use is so common, that it became necessary to find out scientifically the chemical principles responsible for the purgation especially as some phorbols could function as co-carcinogens (Kinsella 1987; Shaofen et al 1991).

In the developing countries there are about 1 billion people living in extreme poverty and vast numbers suffering and dying for want of safe water and medicine, they have no alternative for primary healthcare (WHO 1995).

The aqueous extract of the leaves of *E. heterophylla* is used by the natives to produce purgation. Infact, according to a traditional herbal practitioner when the leaves are used to cook “yam porridge” purgation ensures within 3-4 h after consumption (Sevil et al 1993 and 1994).

Therefore, the objectives of this study is to ascertain the claim by the users of this herb and also to investigate the chemical principle(s) responsible for the laxative activity.

**Experimental**

**Taxonomic identification of plant material.** The plant material (leaves only) was collected at the University of Benin in March 2000 and identified by Prof. L S Gill of the Department of Botany.

A hot aqueous decoction of the leaves of *Euphorbia heterophylla* Linn (Euphorbiaceae) 1.24 kg gave on cooling and defatting with dichloromethane, an aqueous solution which on successively extracting with n-butanol and ethylacetate gave 25.89g and 1.31g of residue, respectively on removal of solvent. The semi-solid extract from the ethylacetate fraction on hydrolysis with dilute tetraoxosulphate (VI) acid gave a yellow powder which on acetylation gave colourless needle clusters identified as quercetin tetracetate. The butanolic fraction had laxative action and contained saponins, phenols, terpenes and diterpenes identified as phorbols but no anthraquinones. The residual aqueous solution contained mainly sugars identified as xylose, maltose, galactose, lactose and lactulose, which are bulkforming laxatives. The purgative action was found to be a joint action of both the phorbols in the butanol fraction and the bulk forming laxative sugars in the residual aqueous fraction.

**Preparation and extraction.** The fresh leaves were collected, weighed and washed with distilled water. 1.24 kg of the fresh leaves of *E. heterophylla* was boiled with 1.5 litres of distilled water for 10 min and filtered then evaporated at 50°C to a syrup liquid by using a rotary evaporator. The total aqueous decoction was first extracted successively with 3 x 200 ml and 100 ml of n-butanol. The residual aqueous extract was extracted with three aliquots parts of 200 ml of ethylacetate. The butanolic fraction was defatted with 200 ml of dichloromethane. The three fractions butanol, ethylacetate and dichloromethane were evaporated to dryness to give 25.902g, 1.312g and 0.858g, respectively. The three fractions were subjected to phytochemical studies.

**Isolation.** 0.470g of the yellow semisolid obtained on removal of the solvent from the ethylacetate fraction hydrolysed with 5ml of dilute tetraoxosulphate (VI) for 30 min. The precipitate obtained (designated FH) was re-dissolved in ethylacetate and subjected to thin layer chromatographic analysis using ethylacetate : n-hexane (9:1). 1H-NMR, 13C-NMR and IR spectrophotometric measurements were obtained.

**Acetylation.** 0.197g of compound FH was acetylated with 5ml acetic anhydride and 3 drops pyridine to give 90.312 mg of colorless needle clusters (designated FA). Compound FA was subjected to 1H-NMR, 13C-NMR and mass spectrophotometric analysis. The melting point was also determined with the kofler melting point apparatus.

**Isolation and identification of the sugars in the extracts.** The cold and residual aqueous fractions after the extraction
ULTRASONIC STUDIES ON SOME AQUEOUS SOLUTIONS OF CARBOHYDRATES AT THREE DIFFERENT TEMPERATURES

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Density, viscosity and ultrasonic velocity measurements have been performed by ultrasonic interferometer technique in some aqueous solutions of three carbohydrates (sucrose, D-glucose and β-D lactose) as a function of molality with different concentration of the order 0.1 to 0.5 mole/kg. At three different temperatures, via 303.15K, 313.15K and 323.15K and at atmospheric pressure, ultrasonic velocity, partial molar volumes, partial molar isentropic compressibility have been calculated and plotted against concentration. The velocity results confirm the conclusions that were originally derived from viscosity data by Einstein that the sugar molecules have a ‘salvation envelope’ attached with a layer of water molecules which decreases with thickness as the temperature of the solution rises. It has been observed that in such solutions it is quite legitimate to look for the dispersion caused by viscosity of the solutions. The apparent molar volume occupied by solute molecules remains constant at one particular temperature, irrespective of the change in concentration of sucrose in water. The compressibility increases slowly as a function of concentration at constant temperature. The data revealed that the compressibility of these different solutions is related with three dimensional hydrogen bond water structure. It is governed by the stereochemistry of carbohydrate. By these studies an overview of the hydration characteristics and the effect of relative position of hydroxyl group within a carbohydrate molecule is given. By increasing the carbohydrate concentration in water the ultrasonic velocity increases, while there is no effect on compressibility of moles. For monomer the apparent molal compressibility depends on the hydration of the mole. The results agree with the previously obtained kinematics data of literature values.

Key words: Carbohydrates, Concentration, Density, Viscosity, Isentropic compressibility, Ultrasonic velocity, Apparent molar volume.

Introduction

The velocity of elastic waves in solution is defined by solute-solvent and solute-solute interactions which are determined by the chemical structure of the solute and solvent molecules. (Nobuo and Kyoshi 1974). However, a clear cut picture of results have been obtained by uncertainties about intrinsic volumes of solute molecules. Still acoustical methods have been developed, with only a minor contribution to the detailed description of the solute-solvent interactions of molecules in aqueous solutions. The successful applications of acoustical methods to the physicochemical investigation of solution became possible after the development of adequate theoretical approach and methods for the precise ultrasonic velocity measurements in small volume of liquid mixture.

Enormous literature is available on the physical and chemical nature of carbohydrates molecules and their structure, most of them are monomers and some are dimmer. Glucose is a dimmer, which is able to rotate about its linkage such that the hydrophobic surfaces were bound through intermolecular forces. These have much interaction for water molecules and it seems reasonable to expect the voids in volume associated with each solute molecule (Paul et al 1999). A group of physicists in USSR (Shel’nikov and Privie 1991) have worked on the bulk elastic properties of glucose, galactose, lactose, sucrose and starch. From their data on ultrasonic velocity and the density of the solutions, the hydration number of carbohydrates in relation to the stereo chemistry have been a subject of study since a long time and due to their importance in our life (Blanshard and Mitchell 1979).

The compressibility of solution can be determined by the effect of solvent, solute and solution. (Roscoe 1952; Mark 1960). The effect of solute molecules can be separated into three parts, the compressibility of solute molecules, solute-solute interactions and the size of the molecule. If the concentration of the solution becomes very low, the second effect becomes negligible (Pyor and Roscoe 1954) measured the ultrasonic velocity of the solution of sucrose and saccharide from the temperatures of 20°C to 80°C and they showed qualitatively that the salvation envelop decreases in size with increasing temperatures, provided the sugar molecules could...
**Pulse Voltammetric Determination of pKₐ of Para-Chloroaniline**

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Monochloroderivatives of aromatic amines are the degradation products of commonly used herbicides. The oxidation potential of monochloroanilines at a number of solid electrodes has been found to be dependent on the number of substituents in the ring system (Suatoni et al 1961). Voltammetric detection of these compounds and their separation in a mixture by liquid chromatography has been reported (Purnell and Warwick 1980; Hart et al 1981). Large amplitude pulse voltammetric experiments suggest that the oxidation reaction of 4-chloroaniline involves the elimination of the one electron as the initial oxidation reaction at the carbon paste anode. The experiments were performed by varying the values of rest potential, drop-time and sweep rate in large amplitude pulse voltammetry. The reasonably constant value of limiting current shows that 4-chloroaniline can be used as a standard for 1-electron oxidation for the investigation relating to the electrochemical studies of selected aromatic amines (Haque 2002). More recent work on these compounds relates to electrodegradation kinetics of p-chloroaniline at solid anodes (Casado et al 1994; Brillas et al 1995). The acidity and basicity of benzene and its derivatives including para-chloroaniline have been treated theoretically in terms of a new quantitative parameter (Feng et al 1995). Effect of potential on the adsorption of p-chloroaniline on silver electrodes has been studied using surface-enhanced Raman spectroscopy (Xu et al 1993). Haloaminobenzenes continue to be the focus of research (Frecero et al 2003). Recently their electrochemistry was reviewed (Haque 2003).

Normal pulse voltammetry has been used to determine pKₐ of p-chloroaniline. The result is in close agreement with the value reported in literature. The procedure outlined in this work represents an example of a new method for the determination of pKₐ of p-chloroaniline.

**Preparation of buffers.** Buffer solutions of the pH range 1.8-6.5 were adjusted to an ionic strength of 0.20 mol/dm³ by addition of the required amount of sodium chloride. The buffer solutions used for electrochemical experiments in specific pH range and their total buffer concentrations were as follow: pH 1.8-3.1, phosphate (0.04 mol/dm³); pH 3.7-5.7 acetate (0.04 mol/dm³); pH 5.7-6.5, phosphate (0.04 mol/dm³). pH was noted by using a digital pH meter and combination glass electrode (Rusling et al 1983).

Solutions of p-chloroaniline were prepared by dissolving the compound in buffers of pH 1.8-6.5, so that the final concentration of p-chloroaniline was 1x10⁻⁵ mol/dm³. Purified nitrogen was bubbled through the solutions for five to ten minutes to remove oxygen and a nitrogen atmosphere was maintained above solutions during voltammetry.

**Carbon paste electrode.** The carbon paste electrode (CPE) was a disc of area 0.12 x 10⁻⁴ m² surrounded by a Teflon collar of diameter 0.3 x 10⁻² m. The carbon paste was prepared from Fisher ACS grade graphite powder, grade no. 38 and nujol as described in literature (Adams 1969). The surface of this electrode was renewed prior to each scan.

**Counter electrode.** A platinum strip, A = 1 x 10⁻⁴ m², or a platinum wire was used as a counter electrode. Reference electrode (silver-silver chloride electrode): A low resistance silver-silver chloride saturated KCl, reference electrode was used for experiments in the aqueous system (Sawyer et al 1995). Voltammetry in a three electrode cell was done using conventional electrochemical instrumentation (Haque 1989). The anodic normal pulse voltammetric behavior of para-chloroaniline was investigated at the carbon paste electrode over the pH range 1.8-6.5. The normal pulse voltammograms for p-chloroaniline in various buffers showed well formed plateaux (Haque 2002). At half the plateaux currents, E½ values were noted in various buffer solutions for the pH range 1.8-6.5. E½ vs. pH plot for 1x10⁻⁵ mol/dm³ para-chloroaniline solutions in these buffers are shown in Fig 1. The first break in E½ vs. pH curve, in Fig 1, corresponds to the pKa value relating to deprotonation of the anilinium species. The value obtained 4.15 is the same as determined using spectrophotometry (Hart et al 1981). The first linear portion of E½ vs. pH plot shows a slope of -58 mV/pH. This value of the slope (Heyrovsky and Vavricka 1972) is consistent with the following equilibrium prior to charge transfer:

\[
\text{p-CIC}_6\text{H}_4\text{NH}_3^+ \leftrightarrow \text{p-CIC}_6\text{H}_4\text{NH}_2 + \text{H}^+ \quad \text{(1)}
\]

Our results thus establish that normal pulse voltammetry affords a reliable route to the determination of pKa value of the reaction noted as in equation (1) above. For comparison, it is mentioned that cyclic voltammetric oxidation of para-chloroaniline at glassy carbon electrode gave pKa value of 5.5 in Briton-Robinson buffers of pH 1.95-12.0 containing 50%...
COMPARATIVE ECOLOGICAL STUDY OF PHYTOPLANKTON. PART II. BAKAR AND PHOOSNA LAKES - PAKISTAN

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A comparative ecological survey of phytoplankton part II of Lake Bakar, district Sanghar and Lake Phoosna, district Badin was carried out during August, 1993 to July, 1996. A total of 122 species belonging to 45 genera of 15 families of 5 orders of class Chlorophyceae were recorded. 11 species were common in both Lakes. 94 species were present in Lake Bakar and 17 in Lake Phoosna. The study showed that the aquatic environment of Lake Bakar is qualitatively much better as compared to Lake Phoosna.

Key words: Phytoplankton, Bakar lake, Phoosna lake.

Introduction

Phytoplankton is an important group of algal flora. These are the producers of food in the food cycle of aquatic ecosystems, fixing energy by the process of photosynthesis. The phytoplankton are widely distributed and are an important component of various ecosystems like marine, rivers, ponds and streams etc. Algal flora is a good indicator of pollution (Patrick and Reimer 1966) and in the water bodies receiving animal, poultry and household waste.

Qualitative and quantitative determinations of phytoplankton are essential for determining the aquatic productivity as they are the chief source of food for aquatic animals including fishes. Bakar lake is subtropical (Blatter et al 1929; Mitchel 1967) and is situated in desert area of Sindh at an altitude of 50 m, latitude 26° 06' North, longitude 68° 10' East. Its width is 2.5 km and length is 45 km. According to Prescott (1961) referring to older, shallow lakes, highly productive for the eutrophic lake so Phoosna is a shallow eutrophic lake, situated in between 68° 55' longitude (East) and 24° 50' latitude (North) at a distance of 20 km from Badin, 5 km, towards north of Hyderabad Badin Road. It is a private owned fishing lake, spread over an area of 500 acres. The lake is shallow, about 2-3 meters deep. Since it is surrounded by agriculture land, consequently also receives leached plant nutrients. The present study was carried out as very little work has been done on the phytoplankton of lakes from Sindh blooms is Kinjhar Lake in summer season (Nazneen 1974).

The present work will give the comparative results of phytoplankton distribution in the Phoosna and Bakar Lakes, where different physico-chemical properties and other parameters have been taken into consideration to study the phytoplankton flora.

Materials and Methods

Phytoplankton were collected monthly from August, 1993 to July, 1996 between 11 a.m. to 3 p.m. with the help of boat using phytoplankton net of 5-10 mm mesh. Water samples were collected using water sampler (Nansen bottle) for studying physico-chemical features using standard methods (APHA 1985) and for quantitative studies of phytoplankton. Samples were preserved in 4% formalin. The species composition was determined by Utremohal method (Lund et al 1958). The microalgae (Ultra nannoplankton) were not counted as Gorham et al (1974), considered these algae comparatively unimportant in high productive lakes. The association of (Ultra nannoplankton) with phyto and tychoplankton so easily collected with the algal net and secondly in polythene bags crush the algal material easily found ultra nannoplankton. Phytoplankton identification and counts were done using inverted light microscope olympus Japan (20X, 40X objective and eyepiece 10X) and identified with the help of available literature (Husted 1930; Majeed 1935; Smith 1950; Prescott 1961; Patrick and Reimer 1966; Tiffany and Briton 1971; Vinyard 1979; Akiyama and Yamagishi 1981; Leghari et al 1997).

Results and Discussion

According to the results of the comparative ecological studies of phytoplankton of Bakar and Phoosna Lakes, qualitative measurement done for the production of phytoplankton is shown in Table 1. Lake Bakar is clearly very productive as...
Assessment of Drinking Water Quality of a Coastal Village of Karachi

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The drinking water quality of a coastal community in Rehri village, Karachi was assessed for human consumption by studying the chemical and microbiological parameters to determine the suitability of domestic drinking water usage. Water samples were collected at household levels from storage tanks and storage containers (earthen jars), main supply line and springs present in the vicinity. Samples collected from different sources indicated that bacterial counts were high for the storage tanks than the earthen jar containers. In storage tanks 71% samples were in high to very high health risk category whereas, in earthen jars 50% samples were in low health risk category. Water samples from two springs showed that samples of Chashma spring had high bacterial count (336 MPN index/100 ml) coupled with high concentration of NO₃ (29.681 mg/l) as compared to Rehri spring (41 MPN index/100 ml, 8.417 mg/l, bacterial count and nitrate concentrations, respectively). All samples collected at household level showed that bacterial contamination exceeded the maximum acceptable concentration. Other parameters (NO₃, NO₂, NH₃, PO₄, free Cl, Ca and Mg hardness) studied including trace elements (Fe, Cu, Cr) in the drinking water were below the WHO drinking water quality guidelines. Fecal coliform, Escherichia coli was also detected including important pathogens Serratia sp. and Enterobacter sp. which were isolated and indicated possible fecal contamination of drinking water at all levels.

Key words: Coliforms, Coastal area, Drinking water, Escherichia coli, Nutrients, Trace metals.

Introduction

The most and widespread health problems in developing countries are water borne diseases, associated with contamination of water either directly or indirectly by microbial or chemical pollutants. The microbial contamination is by human or animal excreta, particularly feces. An estimated 2.2 million deaths in 1998 have been caused by diarrhoea, almost exclusively in the developing countries where safe drinking water is not readily available (WHO 1999) and an estimated 1.3 billion persons do not have access to safe drinking water (UNEP 1996). In Pakistan the access to safe water is available to only sixty one percent population (85% urban, 47% rural). The proper sanitation facility is only available to thirty percent population of which sixty percent are urban and thirteen and half percent are rural (Aziz 1998). In Karachi, a city of more than 10 million people forty percent of the population is living in slums and has limited water and sanitary infrastructure and poor water quality (Anon 2000).

The presence of any living bacteria in drinking water, even in small numbers, indicates, not necessarily a health hazard, but certainly a failure in the chlorination system or recontamination of the water after disinfections. The presence of ‘indicator bacteria’ conventionally known as fecal coliform (Escherichia coli) indicates fecal contamination but it does not prove that water-borne disease is occurring. E. coli are always present in feces; the majorities are not pathogenic, although some strains can cause diarrhoea. In testing untreated water supplies the fecal coliform are most commonly used but other groups of bacteria (such as fecal streptococci) would also indicate contamination. Muneer et al (2001) found variety of fecal coliforms contamination by Streptococcus, Enterococcus, Staphylococcus, Clostridium and E. coli in the drinking water in University of Punjab. Raza et al (1998) studied the seasonal variation of drinking water quality of Northern Areas and Chitral. They found low quality of spring water and the storage vessels (containers) were of high health risk category. Kandhar and Ansari (1998) reported that the Hyderabad dwellers were supplied unchlorinated and contaminated drinking water. Zubair and Rippey (2000) in their evaluation of shallow ground water quality in urban areas of Karachi found presence of inorganic nutrients and bacterial contamination.

Rehri village is one of the oldest coastal villages located in the southeast, 9 km from Quaidabad near Malir in Karachi, on the landward coast of the Korangi creek channel of the Indus River Delta system. The predominant occupation of the people living in this area is fishing with mangrove as a source of fuel wood and fodder. The sources of water supply to the community are from main supply line to household; to share com-
SOME ECOLOGICAL STUDIES ON LINUM USITATISSIMUM LINN

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Introduction

Linum usitatissimum Linn. (Family Linaceae) a medicinal plant has various names such as linseed, flaxseed, common flax, flas, lint (English); alsi, alish, tisi (Punjabi and Urdu); Bazarug, kuman, kuta, tukhme-katan, zaghir, zaghu (Persian). It has many medicinal and other uses (Khan 1969; Khan 1970; Sahid Leitch 1994). Its seeds are used as demulcent in catarrhal complaints and acute or chronic gastritis. Powdered seeds are used as an emollient in poultices for boils, carbuncles, festering sores and other skin affections. The seeds contain galactose, arabinose, rhamnose, xylose, galacturonic and manuronic acids; 30-49% fixed oil, 25% protein, sterols, trilierpenes, 0.1-1.5% cyanogenic glycosides and monoglycosides (Bissent 1994). The cultivation of linseed has several advantages for arable farming in terms of profitability, expanding demands and adaptability of the crop to various soil types and seasons. Several workers have made studies on its cultivation (Smid 1998) and under different soil moisture levels (Teo et al 1989; Lambert et al 1990; Singh and Sharma 1991; Ranney and Bir 1991). The present study was conducted to see the effect of different soil types, light conditions, fertilizers and water levels on the growth performance of this plant.

Materials and Methods

Seedlings of L. usitatissimum were raised in nursery beds with loamy soils on October 30, 1998. At an average height of 5 cm, 10 seedlings were transplanted to equal size (18 x 22 cm) pots containing loamy soil. After 7 days, they were thinned to 5 uniform seedlings per pot. There were 5 replicates for every treatment in each of the experiments. In all the experiments loamy soil was used except where the effect of soil type was to be investigated. Weeding was done by hand. The pots were maintained at field capacity (Hussain et al 1989) during the experimental period except in experiment where the water and different levels of soil moisture was tested.

To see the effect of soil types plants were grown in similar pots with equal volume of sandy, clayey or loamy soils. The pots were kept in open uniform condition in net house and maintained at field capacity (Hussain et al 1989). The effect of fertilizers was determined by applying urea, diammonium phosphate (DAP) and organic matter in each pot following Jalis and Khan (1982). The organic matter was added @ of 0.05% to each pot. The pots were placed in full sunlight, partial light and shade to see the effect of different light conditions (Mubarak et al 1983).

To determine the effect of soil moisture on plant growth the pots were maintained at field capacity (control condition), water stressed and waterlogged condition. Initially all the pots were saturated. The water stress was created by withholding the water till the temporary wilting was evident. They were rewatered and again allowed to reach the temporary wilting point. In this way four stress cycles were run. Pots subjected to waterlogging were kept saturated all the time.

In all the experiments, the data on growth behaviour such as number of leaves, branches, height of plants, number of flowers, number of fruits and seeds were taken on April 20, 1999. The amount of total chlorophyll, a and b of shoots was determined following the method of Hussain (1989). The results were subjected to t-test.

The application of fertilizers appeared to have no significant effect on the overall growth and productivity. Various salt types affected various parameters with a tendency of better growth in light textured soils. The plant grew better in full light condition with optimal soil moisture. The plants wilted to death under shady condition. Both the water stressed and waterlogged conditions reduced the growth performance but the waterlogged condition adversely affected the plants. Therefore, it is concluded the L. usitatissimum could be grown in semi-arid condition on marginal lands.

Key words: Linum usitatissimum, Soil moisture, Growth, Productivity.

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Sera from Nigerian Children with Genitourinary Schistosomiasis Having Immune Complexes and Heat Labile Leucocyte Migration Inhibitory Factors Without Impaired Cellular Immunity

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Introduction

Most studies on Schistosoma haematobium are on the level of epidemiology, pathology, biology, control and prevention. However, immunology of genitourinary schistosomiasis in relation to urinary egg count among Nigeria school children has not been fully studied. Available information on the immunological studies of urinary schistosomiasis by Lucas and Boros (1992) and Newar et al (1992), on experimental animals are inconsistent and focused attention on a few indices. The belief that immunity is a major factor controlling the prevalence and intensity of schistosomiasis in men is a deep seated one. It seems impossible to most observer that subjects in endemic areas who are exposed to infected waters are not constantly re-infected. Furthermore, there is a continuous trickle of anecdotal evidence of the infectivity of the parasite supplied by case reports of individual from non-endemic areas who became infected after only a short term exposure (Warren 1973).

During infection patients undergo immunologic modulation, which results to decrease inflammation around the eggs, and this allows patients to survive up to chronic stage of infection (Boros et al 1975). The evasion of penetrating schistosomula from the destruction of eosinophils and non-specific antibody of the host activates the macrophage, as further defensive mechanism (Olds and Ellner 1984). The importance of macrophage in schistosome infection derides from increased secretion of granule-poietic colony stimulation factor (CSF) from monocyte-macrophage and lymphocytes (Bolin and Robinson 1977; Verma et al 1979). Schistosomiasis is associated with a significant decrease in the bacteria-phagocytic
**Preliminary Investigation on the Herbicidal Potentials of the Extracts from Maize Inflorescence on Seeds of Three Tropical Compositae Weeds**

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The herbicidal potentials of extracts derived from maize inflorescence were collected at 48, 96, 144, 196 and 240 h after formation were examined on the weeds, Siam-weed (*Chromolaena odorata*), Node-weed (*Synedrella nodiflora*) and Tridax (*Tridax procumbens*). Two sets of aqueous extracts (A and B) were prepared. Treatment A involved the dispersion of the powdered extracts from the inflorescence in distilled water, while treatment B involved the addition of sodium chloride to the dispersed powder in distilled water. The extracts inhibited growth of the seeds of the weeds used when compared to the control. In both treatments 48 h extract tends to be the most effective. The effectiveness of the extracts decreases with an increase in the age of the inflorescence. Hence, more extract-treated seeds germinate with an increase in the age of the inflorescence. The addition of sodium chloride to the extracts tends to increase the potency of the extracts in delaying germination of weed seeds.

**Key words:** Herbicide, Inflorescence, Weeds.

**Introduction**

The loss incurred as a result of weeds infestation on farms has made the invention of herbicides the miracle of the last millennium. Unfortunately, most of the formulations have low biodegradability rates (Adesina et al 1998) hence were detrimental to the flora and fauna species of the environment (Taylor 1998). Also, herbicides are expensive and often beyond the reach of resource of poor farmers who invariably constitute the major stakeholders (Arendsen et al 1996).

The search for a sustainable approach to weed control technique that would be environmentally friendly and readily accessible to the resource-poor farmers has now been the focus of ecologists and environmental scientists (Akoroda 2000; Chikoye 2000). Recently, Trebuil (2002) reported the development of a household weed killer by the paddy farmers in northern Thailand. The present study is a part of the ongoing attempt to develop a sustainable weed control method from materials that would be accessible to the stakeholders for growing crops in the field.

**Materials and Methods**

Maize (*Zea mays*) inflorescences were collected at 48, 96, 144, 196 and 240 h after formation. They were ground in a mortar and later blended with powders in an electric blender. Portions of 6 g each of the 48, 96, 144, 196 and 240 h inflorescence powders were measured out. Each of the portions were dispersed in 100 ml of distilled water. The mixtures were then filtered using Whatman No.1 filter paper and the filtrates used for the experiment named as treatment A.

Similary, Portions of 5 g each of the 48, 96, 144, 196 and 240 h inflorescence powders were measured out. Each portion was mixed with 1 g sodium chloride (NaCl, common salt) and dispersed in 100 ml distilled water. The mixtures were then filtered and the filtrates used for the experiment named as treatment B.

Matured weed seeds of *C. odorata*, *S. nodiflora* and *T. procumbens* were collected from the campus of the University of Ado-Ekiti. Two sets of Petri dishes were double layered with Whatman No.1 filter papers. A set of the Petri dishes that consists of 75 Petri dishes were used for treatment A and the other set of 75 Petri dishes for treatment B.

Each set of the Petri dish was divided into 3 sub-sets of 25 Petri dishes each. A sub-set was used for each weed. Five seeds of each of the weeds were placed in each Petri dish and moistened daily with filtrates of the 48, 96, 144, 195 and 240 h of treatments A and B.

Control experiments were set up for the two treatments without adding the extracts. Control experiments for treatment A were moistened with distilled water. In treatment B, 1 g of NaCl was dissolved in 100 ml of distilled water and the resulting solution was used for the control experiments. Thus, each of the extract treatments and the control were replicated five times.
Effect of Aqueous Extract of Avicinnia Marina on Myocardial Contraction of Isolated Mammalian Heart

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Aqueous extract of Avicinnia marina commonly known as mangrove showed a marked inhibitory action on isolated mammalian heart in vitro. The degree of inhibition was found to be highly dose dependent. A dose of 100 mg/kg caused cardiac arrest for few seconds. The force and magnitude of cardiac contractions were regained to pre-injection level after 1.6-2 min. Pretreatment with atropine was found to have no effect on myocardial contractions. The amplitude of cardiac contractions was reduced slightly after the administration of adrenaline.

Key words: Avicinnia marina, Mangrove, Mammalian heart.

Materials and Methods

Collection and identification. Mangrove plant was collected from the coastal areas of Karachi, identified by a taxonomist and a voucher specimen was kept in our laboratory under PCSIR Herbarium No. 585 for future reference.

Preparation of extract. The collected plant material was washed thoroughly first with tap water and then with distilled water. Dried in air at room temperature and then chopped into small bits. 1.0 kg of chopped material was soaked in 95% ethanol for a period of 5 days with continuous stirring for 6 h per day. Solvent was then decanted and concentrated under reduced pressure at room temperature. The resultant gel like alcoholic extract was partitioned with (V/V 2:1 ratio) water and pet ether with vigorous shaking in separating funnel. The resultant aqueous layer thus formed was separated and concentrated to maximum dryness in rotary evaporator. The mass thus obtained as aqueous extract was used for the study.

Standard reference solutions. Adrenaline, acetylcholine and atropine were used as standard references. These solutions were made in sterile distilled water.

Test solution. Mac. Ewen’s solution (Robert 1971) having the following formula was used to perfuse the isolated heart.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>7.60 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.24 g</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.19 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>2.10 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.00 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.50 g</td>
</tr>
</tbody>
</table>

Double glass distilled water ———Vol. made to 1 liter.
**Introduction**

Lesser grain borer, *Rhizopertha dominica* is a cosmopolitan insect and a serious pest of stored grains especially wheat and flour. According to our food requirements wheat has a prominent place in our daily diet. In Pakistan farmers store about 60% of wheat for food and for sowing requirements. Unfortunately about 5-7% of wheat is destroyed by insect pests.

The insect also attacks the germ part of the grain, feeds upon the endosperm and fills the burrows with excrement which leads to poor quality of seed. In Karachi, the lesser grain borer damages more wheat in Dockyard areas probably due to damp climate which softens the hard pericarp of wheat grain. Conventional (synthetic) pesticides are used for the control of this insect pest. But their indiscriminate use causes problems like pollution, residual toxicity and insect resistance (Zettler 1982; Holiday *et al.* 1988; Saleem and Shakoori 1993). Synthetic pyrethroids are rapid in action, have low mammalian toxicity and wide controlling range (Bagherwal *et al.* 1994). Several workers have reported effect of biopesticides on certain enzyme levels of insects (Bandyopadhyay 1982; Hoyaoka and Dauterman 1982; Shakoori and Saleem 1989; Shakoori *et al.* 1994, Ahmad *et al.* 2001).

Plant products kill insects, however, they have low mammalian toxicity, leave no toxic residues and do not pollute the environment.

Extracts from dumdum leaves (*Clerodendrum inerme*) can be used effectively to protect stored grains from insect infestations. The extract possesses anti-feedent, repellent, insecti-cidal and growth disrupter effects on insects. There are no estimates of dumdum leaves production in Pakistan. However, it is widely grown in Sindh and Punjab and some farmers are aware of its pest control properties.

As the synthetic insecticides are toxic and hazardous, so phytopericides which are much less toxic and biodegradable, the present extract was used against *R. dominica*.

**Materials and Methods**

*Rhizopertha dominica* beetles were obtained from PARC, TARC, University Campus, Karachi, reared under controlled conditions i.e., 28±3 °C and 65±5% R.H. Sterilized wheat was used as rearing medium. All experiments were carried out with 7 days old adults of uniform size. Cyhalothrin was purchased from market and extraction of *Clerodendrum inerme* leaves was prepared in the laboratory.

**Extraction of sample.** 250g *C. inerme* leaves, collected from Karachi University Campus, were washed for the preparation of extract. Leaves were macerated in 50% methanol (1:1 H₂O: CH₃OH). The macerated leaves were left for 24 h in 250 ml of 50% methanol. Maceration of leaves was done in Ultra Turax grinder and homogenized for 30 min. Finally, it was filtered twice and stored in a refrigerator at 10°C.

For the treatment of insects filter paper impregnation method was employed. After preliminary test 236, 475, 950, 1900 and 3800 µg/cm² doses of *C. inerme* and 0.000316, 0.000633, 0.00126, 0.00253 and 0.00507 µg/cm² doses of cyhalothrin were selected. These doses were applied on the filter paper of 2.5 cm diameter with the help of pipette. Twenty *R. dominica* adults of
**INFLUENCE OF DIFFERENT TYPES OF MILK AND STABILIZERS ON SENSORY EVALUATION AND WHEY SEPARATION OF YOGHURT**

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The influence of seven different stabilizers i.e. pectin, guar gum, carboxymethylcellulose (CMC), carrageenan, sodium alginate, corn starch and gelatin was studied at 0.4% levels in buffalo milk with 16.6% total solids, cow milk with 13.5% total solids and mixture (1:1) of both having 15.0% on syneresis, body/texture, flavor, acidity and color in yoghurt. Results showed that corn starch gave best results in controlling syneresis in yoghurt followed by gelatin, pectin, sodium alginate, carrageenan, guar gum and CMC in buffalo milk as compared to mixture and cow milk. Treatment (T19) having 0.4% corn starch and 16.6% total solids got maximum scores in flavor, body/texture, acidity and appearance than all other six stabilizers. This sample had firm coagulum, less separating whey, good aroma, pleasant taste and rheologically superior to all other samples. Statistical analysis showed that the treatments, storage intervals and total solids had a significant effect (P<0.05) on syneresis, body/texture, flavor, acidity and color of the yoghurt samples.

**Key words:** Yoghurt, Milk stabilizer, Syneresis.

Whey separation or syneresis is a major problem of yoghurt which occurs when the body of yoghurt is cut and undesirable watery (whey) comes on the surface of yoghurt. Different stabilizers are used to overcome this problem during processing and storage of yoghurt. Stabilizers (sometimes referred as hydrocolloids) have two types of action i.e. the binding of water and increase in the viscosity in yoghurt (Boylw 1972). The stabilizers permitted by FAO/WHO in 1976 are natural gums including plant extracts (pectin), seed flour (guar gum), cellulose derivatives (CMC), seaweed extracts (carrageenan and sodium alginates) and cereal starches (corn starch). From animal source includes gelatin (Glicksman 1979).

The most common inoculating material used by the modern dairy plants is the culture comprising of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the ratio of 1:1, available either in powder or in tablet form. These grow together symbiotically and are responsible for the production of good taste and aroma in yoghurt. This fermentation process also causes pre-digestion of protein, carbohydrates, fats, increase in B–vitamins, enzymes and enhance the calcium bio-availability (Shahani 1983; Kaup et al 1987). So far little research work has been conducted on the effect of stabilizers on the physico-chemical characteristics, particularly on syneresis of yoghurt. It is the continuation of our previous study (Ayub and Siddiq 2003), which has been undertaken to improve the quality of yoghurt by controlling whey separation with added stabilizers in fresh dairy farm milk of buffalo and cow (1:1 and individual), available to common consumer in any season.
COMPARISON OF HYPER PRODUCER ASPERGILLUS NIGER CULTURES (IFS-5, IFS-6 AND IFS-17) FOR CITRIC ACID FERMENTATION IN SURFACE CULTURE

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Citic acid fermentation by Aspergillus niger is an aerobic process and the organism needs a fairly high and constant oxygen supply for its growth (Hang 1988; Haq \textit{et al} 2000). Surface culture technique (SCT) is a conventional method of citric acid production. Most of the pilot plants are using this technique due to low energy consumption and manpower involved (Singh \textit{et al} 1998). In SCT, the substrate remains stationary and organism form mycelial mat on the surface of medium. The relation between constitution of the fermentation medium and rate of citric acid production has been investigated (Elimer and Ewaryst 1995). Sucrose salt medium as synthetic fermentation medium while cane or beet molasses as natural fermentation medium have long been employed as usual routine basal media (Ali \textit{et al} 2001). Clark \textit{et al} (1965) obtained 80\% conversion of available sugar, 8 days after incubation. Farouk \textit{et al} (1977) pointed out that the age of culture also affect the yield of citric acid. Both of these authors used synthetic medium in their study on citric acid fermentation. The mutant strains have greater ability to produce citric acid. The present investigation deals with the time course study during citric acid production by surface culture technique using three different mutant cultures of \textit{A. niger} (IFS-5, IFS-6 and IFS-17) and their comparison on kinetic basis.

Organism. In the present study, 3 different mutant strains of \textit{A. niger} (hyper producers of citric acid) were used. These strains (IFS-5, IFS-6 and IFS-17), have already been developed by mutation (Ali \textit{et al} 2001) in Biotechnology Laboratories, Government College University, Lahore and maintained on potato dextrose agar medium. The cultures were stored at 4\(^\circ\)C in a refrigerator.

Culture medium. Sucrose salt medium containing (g/l); sucrose 150.0, MgSO\(_4\).7H\(_2\)O 0.25, KH\(_2\)PO\(_4\) 2.5, NH\(_4\)NO\(_3\) 2.5, pH 3.5 was used as the basal fermentation medium.

Analysis. Dry cell mass was determined according to Kirimura \textit{et al} (1992). Residual sugar was estimated by DNS method (Ghose and Ghen 1970) while pyridine acetic anhydride method was employed for the determination of citric acid as reported by Marrier and Boulet (1958). The kinetics of time course was also undertaken (Pirt 1975).

Time course study is one of the most critical factors, which determines the efficacy of the process along with product formation (Elimer and Ewaryst 1995). The data of Table 1 shows the biosynthesis of citric acid at different intervals of time. Three different mutant strains of \textit{A. niger} (IFS-5, IFS-6 and IFS-17) were used for their time course comparison. These cultures were incubated at 30\(^\circ\)C for 1-11 days. The maximum production of citric acid (48.14 g/l) with mutant IFS-5 was obtained 10 days after incubation, which seems to be uneconomical due to longer fermentation period. When IFS-6 was used for inoculating the culture medium, a maximum citric acid production of 31.52 g/l was obtained with a high degree of consumable sugars (96.0 g/l). Although the fermentation period became very short (5 days) as compared to mutant IFS-5 but the yield of product was too low for an economical process.

The maximum production of citric acid (46.22 g/l) by mutant strain of \textit{A. niger} IFS-17, was achieved 7 days after the inoculation. The dry cell mass and sugar consumed were 23.20 and 94.0 g/l, respectively. Further, increase in the incubation period did not enhance the production of citric acid, rather it was decreased. It might be due to the reduction of sugar contents in the fermentation medium and accumulation of other by-products. Thus incubation period of 7 days was found to be optimum for maximal citric acid biosynthesis. Our results are in agreement with the findings of many workers (Jaszwry \textit{et al} 1971; Singh \textit{et al} 1998). For maximum citric acid production, the optimum time of incubation varies from organism to organism depending on fermentation medium provided (Elimer and Ewaryst 1995).

The kinetic study of time course during citric acid fermentation by mutant strains of \textit{A. niger} was also worked out. There was a marked difference of product yield coefficients (Yp/s and Yp/x) among different mutant strains i.e., maximum Yp/s value in case of IFS-17 (0.492 g/g) was much higher as compared to mutants IFS-5 and IFS-17 (0.384 and 0.328 g/g).