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Digital simulation of cyclic voltammogram of ruthenocene in benzonitrile suggests that electrooxidation of ruthenocene at platinum electrode involves a one-electron oxidation. The ruthenium cation presumably combines with ruthenocene in a fast following chemical reaction. The proposed dimeric monocation so formed shows a one-electron electroreduction at a much lower potential.

**Key words:** Simulation, Cyclic voltammetry, Ruthenocene

**Introduction**

Digital simulation has been found very useful in a number of complex electrochemical problems involving complicated kinetic schemes and non-uniform current distribution at the working electrode (Bard and Faulkner 1980). Electrochemical oxidation of ruthenocene employing a mercury anode has been reported (Hendrickson et al. 1972).

\[
\left[\eta^6-C_6\text{Me}_6\right]_2\text{Ru}^{2+}\n\]

undergoes a two electron reduction with a change in the coordination mode of one of the arene ligands. Digital simulation was used in a recent study to confirm that the structural change occurred during the second electron transfer reaction and to investigate the effect of the solution electron transfer reaction. These simulations are studied in this capsule using DigiSim (Digisim 2003).

The electrochemistry of 1,1-bis(diphenylphosphino)ferrocene (dppf) derivatives of Ru \(_3\) (CO) \(_{12}\) was investigated. Two known compounds [Ru \(_3\) (CO) \(_{12}\)(m-dppf)] \(_2\) (1) and [Ru \(_3\) (CO) \(_{12}\) dppf] (2) and a new compound [Ru \(_3\) (CO) \(_{11}\)(m-dppf)Ru \(_3\) (CO) \(_{11}\) ](3) were prepared. The reductive electrochemistry of (1) and (2) showed an irreversible reduction and a follow-up oxidation, similar to Ru \(_3\) (CO) \(_{12}\). The electrochemistry of compound (3) showed two irreversible waves and a follow-up oxidation. The oxidative electrochemistry of (1-3) showed a dppf-based chemically reversible wave, and an irreversible wave similar to that of Ru \(_3\) (CO) \(_{12}\). Trends were also noted between the oxidation potential and the number of coordinated phosphorus atoms (Nataro 2003).

Splitting of a single two electron cyclic voltammetric wave for bis-arene complex (\(\eta^6-C_6\text{Me}_6\) \(_2\) Ru into two one-electron components involving an EE (consecutive electron transfer) mechanism has been reported (Pierce and Geiger 1989). The electrochemical reduction of \([\eta^6-C_6\text{Me}_6\) \(_2\) Ru][BF\(_4\)]\(_2\) has been shown to occur into two, one-electron steps, each manifesting solvent-dependent formal potentials. The heterogeneous charge-transfer kinetics varied with electrode material. Cyclic voltammetry (CV) was found to be sensitive to homogeneous reaction, which occurred both within the electrode reaction layer, and in the bulk of solution. Whereas, the reduction waves of \((C_6\text{Me}_6)\text{Ru}^{2+}\) were resolved in methylene chloride and separated by -0.14 V (E\(_o^2\) - E\(_o^1\), E\(_o^2\) = -1.45 V vs. Fe/Fe\(^+\)), only a single two-electron wave was observed in acetonitrile because of a negative shift of E\(_o^1\) with respect to E\(_o^2\) (E\(_o^2\) - E\(_o^1\) = +0.03 V, E\(_o^2\) = -1.40 V). Both reductions displayed Nerstian behavior at mercury electrodes. However, the Ru(I/0) couple showed quasi-reversible charge-transfer kinetics at platinum disk electrodes. At platinum, the single two electron wave was found to split into two, one-electron components over a range of sweep rates, which varied with analyte concentration. The Ru(I) complex was also subject to a follow-up reaction having a rate constant of 1.0 s\(^{-1}\). Detailed explicit finite difference simulations of the CV curves allowed solution of the electron transfer parameters for the two, one-electron couples in acetonitrile at platinum electrodes. Homogeneous cross-section rates for electron transfer between Ru(II) and Ru(0) were estimated. Synthesis and
**Drug Release Profile of Malic Acid-Phthalic Acid Butane 1,4-Diol Copolyester**

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(Received July 30, 2003; accepted April 20, 2004)

Five copolyesters (I-V) in varying mole ratios of malic acid and phthalic acid were separately synthesized with 1 mole of butane 1,4-diol using p-toluene sulfonic acid (0.4% of the total weight) as catalyst under vacuum at 120-125 °C for about 6 h. The malic acid-phthalic acid-butane 1,4-diol copolyesters (MPBC) were characterized by their IR spectra, molecular weight, elemental analysis and solubility behavior in common organic solvents. The polymer III had the highest molecular weight and it was selected for subsequent experiments. Its hydrolytic degradation study in solutions of different pH values showed that it remained intact in solutions of pH values 1.2-6.0, but gradually degraded in solutions of pH values >6.0. The drug delivery profile of MPBC as an enteric coating material was investigated in simulated gastric fluid (pH 1.2) and then in simulated intestinal fluid (pH 7.4), it was found that the drug release pattern did not conform to enteric coating requirements. In the case of matrix tablets where drug was dispersed in the MPBC, it was found that the drug delivery was zero order up to 12 h releasing 88.0% of diclofenac sodium and up to 13 h releasing 86.50% of naproxen, afterwards release of drugs was negligible.

**Key words:** Malic acid, Phthalic acid, Copolyester, Drug delivery profile.

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**Introduction**

In recent years, considerable interest has grown to synthesize biodegradable polymers for medical and agricultural uses, because such degradable carriers have the advantage of eliminating the necessity of their removal. Many of the existing biodegradable carriers are linear polymers (Heller 1980) such as polylactic acid, polyglycolic acid and their copolymers (Yolles et al. 1975) which are biodegradable and are being used for specialized application such as controlled release drug formulation (Graham 1978; Rosenberg et al. 1983), insecticide and pesticide carriers as well as non-toxic surgical implant materials. A large number of polymers have a built-in self-destruct mechanism by which they undergo slow hydrolytic and microbial degradation releasing the impregnated material at controlled rates. Matrix tablet is one of the least complicated approaches to the manufacture of sustained release dosage forms, which consists of a drug dispersed in a polymer, the polymer playing the role of a matrix (Touitou and Donbrow 1982; Bidah and Vernaud 1991). Cellulose acetate phthalate, hydroxypropyl methylcellulose have been used as enteric coatings (Madan 1990). Aliphatic polysters could display an excellent biocompatibility and be degraded in most biological environments. Biodegradable polymers from glycolic acid (PGA) or DL- lactic acid (PLA) are the simplest linear aliphatic polysters, which are currently the most widely used synthetic, degradable polymers in human medicine (Engelberg and Kohn 1991; Ouchi et al. 2000). Keeping the same view ahead, malic acid-phthalic acid-butane 1,4-diol copolyester (MPBC) has been synthesized and it has been investigated as a carrier for sustained and controlled release of drugs.

**Experimental**

Malic acid, phthalic acid and butane 1,4-diol were the monomers of the synthesized copolyesters and were purchased from Sigma Chemical Co. England, E. Merck, India Ltd. and BDH, England, respectively. Core tablets of diclofenac sodium (50 mg) supplied by Chemico Laboratories, Rajshahi and naproxen (50 mg) by Beximco Pharmaceuticals Ltd. Tongi, Dhaka, were of analytical grade. Reference standard of diclofenac sodium (DS) (99.2% Purity) and naproxen (99% purity) used for analytical purpose were obtained from Beximco Pharmaceuticals Ltd. Tongi, Dhaka, Bangladesh.

**Synthesis of the polymer.** Five mixtures of malic acid and phthalic acid in different mole ratios were taken separately with 1 mole of butane 1,4-diol along with p-toluene sulfonic acid (approximately 0.4% of the total weight) as catalyst in a 100 ml beaker and were allowed to undergo polycondensation in a reaction vessel under vacuum at 120-125 °C for about 6 h. The solid polymers were then collected from the reaction*Author for correspondence.*
**FUSED PYRIMIDINES: PART-I: SYNTHESIS OF IMIDAZO[1,2-a]THIENO[2,3-d]-PYRIMIDIN-5(1H)-IMINE AND PYRIMIDO[1,2-a] THIENO[2,3-d] PYRIMIDIN-6-IME**

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(Received November 11, 2003; accepted April 27, 2004)

Annelating reagents, 2-(methylthio)-2-imidazoline (2) and 1,4,5,6-tetrahydro-2-methylthiopyrimidine (4) were prepared from 1,2-diaminoethane and 1,3-diaminopropane via 2-imidazolidinethione and 1,4,5,6-tetrahydropyrimidin-2-thione respectively. The substrate, 2-amino-4,5-dimethylthiophen-3-carbonitrile (5) was prepared from butanone. The reaction of substrate (5) with the annelating reagents, (2) and (4), in HMPT led to 2,3-dihydro-6,7-dimethylimidazo[1,2-a]thieno[2,3-d]pyrimidin-5(1H)-imine (6) and 1,2,3,4-tetrahydro-7,8-dimethylpyrimido[1,2-a]thieno[2,3-d]pyrimidin-6-imine (7) in good yields.

**Key words:** Annelating reagents, Substrate, HMPT, Fused pyrimidines.

**Introduction**

The most important and naturally occurring diazines are pyrimidine bases uracil, thymine and cytosine, which are constituents of the nucleic acids (Blackburn and Gait 1996). Following from this, several pyrimidine nucleoside analogues have been developed as antiviral agents, for example idoxuridine is used in the treatment of Herpes infections of the eye and AZT (Zidovudine) is the most widely used anti-AIDS drug; 3-TC (Lamivudine) is used to treat both hepatitis B and AIDS, while d4T (Stavudine) is a fourth drug approved for treatment of HIV infection and AIDS. The pyrimidine ring also occurs in the vitamin thiamin (Joule and Mills 2000). Derivatives of thieno[2,3-d]pyrimidine system are of great interest because of their antimalarial (Albert 1986), antibacterial and antifungal (Rahman et al 1999 & 2003) activities. (Hetero) Aromatic o-aminosteres and o-aminonitriles undergo ready cyclization, which allows convenient preparation of a variety of condensed pyrimidines (Taylor 1987). Thioseposeudourea is a versatile reagent for the preparation of fused pyrimidines (Sauter et al 1997). Along this route and in continuation of our ongoing program (Chowdhury et al 2000 a & b and 2001), we report here the synthesis of 2,3-dihydro-6,7-dimethylimidazo[1,2-a] thieno [2,3-d] pyrimidin-5 (1H)-imine and 1,2,3,4-tetrahydro-7,8-dimethylpyrimido[1,2-a]thieno [2,3-d] pyrimidin-6-imine, (6) and (7), respectively.

**Experimental**

Melting points were determined in open capillary tubes and are uncorrected. 1H-and 13C-NMR spectra were recorded on a Bruker AC 200 spectrometer using DMSO-d6/CDCl3 as solvents and TMS as an internal standard (chemical shifts in δ, ppm). TLC was run on silica gel-G plates and spots were located by iodine vapor. All evaporations were conducted under reduced pressure at bath temperature below 50°C. The starting material (2) (Dave et al 1988), (4) (Gewald et al 1966) and (5) (Chowdhury 1996) were prepared according to reported in literature.

2,3-Dihydro-6,7-dimethylimidazo[1,2-a] thieno [2,3-d] pyrimidin-5 (1H)-imine (6). A solution of o-aminonitrile (5) (0.465 g, 3 mmol) and 2-methylthio-2-imidazoline (2) (0.522 g, 4.5 mmol) in hexamethyl phosphoric triamide (HMPT, 6 ml) was heated under reflux at 160°C for 3 h. After cooling to room temperature, crushed ice (35 g) was added and the mixture stirred for additional 1 h. The separated solid was collected by filtration and recrystallized from methanol to give (6) as brown crystals, m.p. 177-178°C, yield 0.47 g (70%). Anal. Calc. for C10H12N4S (220.30): C, 54.52; H, 5.49; N, 25.43; Found C, 54.30; H, 5.50; N, 25.08%.

1H-NMR (CDCl3): δ7.40 (s,1H,NH), 6.60 (bs, 1H, NH), 3.90 (t, 2H,3-H), 3.60 (t,2H,2-H), 2.46 (s, 6H, 2CH3). 13C-NMR (DMSO-d6) δ160.44, (s, C-5), 155.02 (s, C-8a), 129.37 (s, C-7), 124.60 (s, C-5a) 111.96 (s, C-6), 42.54 (t, C-3), 39.65 (t, C-2), 19.25 (q, 7-CH3), 18.06 (q, 6-CH3).

1,2,3,4-Tetrahydro-7,8-dimethylpyrimido[1,2-a]thieno[2,3-d] pyrimidin-6-imine (7). This compound was prepared from o-aminonitrite (5) and 1,4,5,6-tetrahydro-2-methylthio pyrimidine (4) following the same method used for the preparation of (6) to give (7) as brown crystals in 64% yield, m.p.>250°C. Anal. Calc. for C11H14N4S (234.33): C, 56.38; H, 6.02; N, 23.91; Found C, 56.15; H, 6.03; N, 23.58%.

*Author for correspondence*
Introduction

Large quantity of ice is presently being used in Karachi, the most populated city of Pakistan, having a population above twelve million, by high as well as low-income groups and also by snack bars, restaurants and other commercial outlets in the local tropical climate which prevails almost throughout the year. Bulk quantity of ice is also used to preserve meat, fish and other perishable edible and non-edible items during the summer season. Although ice is considered as a food and its manufacturing is covered by the regulations for food, it has been observed that ice making is not properly regulated and that there is no proper control of the relevant authorities on the quality of water being used for the production of ice in Karachi. In the absence of any precautionary measure, water is at present being indiscriminately used from all available sources in ice making factories spanning through the whole Karachi city.

The population of the mega city of Karachi has multiplied several fold during the last two decades. Owing to the unprecedented increase in the population density and poor planning on the part of administrators and town planners, great pressure has been built up on the inherent infrastructure and above all on provision of essential amenities to the local population. As a result, the whole system laid down for supply of water and disposal of sewerage has been over burdened and badly crumbled (Mahmood et al 1998). Water, if contaminated by pathogenic organism, may pose serious health risks and is the root cause of several water borne diseases (Moe et al 1991; Falco et al 1993). The ground water is usually considered clean and safe, having constant chemical composition but unlike surface water, it is neither renewable nor self cleans- ing (Anonymus 1990). The quality of ground water in the Karachi region has been badly affected, both chemically as well as microbiologically, due to the mixing of sewage wastewater from leaking sewage lines (Zubair and Rippey 2000). The microbiological quality of piped/supply water in Karachi is also poor, because the water supply system provides intermittent service and usually laying of the pipelines is faulty. The treated supply water is often contaminated due to admixture with seepage from sub soil water and sewers (Beg et al 1985).

Surveillance and monitoring of ice quality to ensure microbiological and chemical safety, are vital public health functions especially in developing countries like Pakistan because human illness is also attributed to ingestion of drinking water, beverages and juices served with contaminated ice.

As no evidence could be found that the quality of ice being manufactured and sold in local market of Karachi has been examined, this study was carried out to determine the quality and source of water being used for ice making and to assess

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**Quality of Ice Manufactured in Karachi City**

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(Received September 17, 2003; accepted June 2, 2004)
STUDIES ON THE UTILIZATION OF SPENT CATALYST USED IN OIL REFINERY

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Enormous amount of the spent cobalt-molybdenum/alumina catalyst, used for the hydrodesulphurization process in the oil refineries, is locally available for its safe disposal. Studies were undertaken on this spent catalyst for the extraction of valuable metals contained in it. Its chemical evaluation indicated the presence of CoO 3.43%, MoO3 14.50%, Al2O3 79.67% and SiO2 2.40%. In view of the high prices of cobalt and molybdenum in the international market, the research and development work were mainly concentrated on the extraction of these metals. Leaching studies undertaken on the samples indicated the optimum recovery of cobalt up to 96% and 96.5% for molybdenum.

Key words: Catalyst, Hydrodesulphurization process, Leaching, Extraction.

Introduction

A typical hydrogenation process developed for the refining of petroleum distillates is the cobalt-molybdate process (Berg et al 1947; Byrns et al 1943). The process is applicable to the desulphurization of both straight-run and cracked distillates. Cobalt-molybdenum/alumina catalyst is used for the Hydrodesulphurization process in oil refinery.

The catalyst contains metallic oxides and can be used for a certain definite length. It is used in the presence of hydrogen from catalytic reforming operations. Because the charge stock is liquid, heavy feedstock can be treated. The sulphur content reduces from 2.9% to 0.01% in the fraction boiling less than 430°F to 0.43% in the fraction boiling over 500°F (Hoog et al 1953; Vladimir and Kenneth 1956). With the passage of time, the activity of the catalyst is reduced and it has to be replaced with a new stock during plant shutdown/turnaround. Before this, used catalyst is replaced with a new one, it is generated to burn off all sulphur and carbon content to make it safe for storage and transportation through no extra precaution/packing.

Tonnes of the spent catalyst need safe and economical utilization without creating the environmental problem and severe health hazards by its prolonged exposure, if swallowed or inhaled. It may also cause sensitization by skin contact.

The chemical composition of the catalyst comprises the oxide of cobalt, which varies from 1-10% and the oxide of molybdenum from 10-20% with a balance amount of alumina indicating the potential for the extraction of these materials from the spent catalyst. Studies had been undertaken on the recovery of cobalt-molybdenum-alumina by Grzechowiak et al (1986). The present studies were aimed at for the better recovery of these metals rendering it feasible.

Experimental

Sample preparation. The catalyst sample was in the form of beads with an average size of 2.5x5.0 mm. The material was subjected to grind of an appropriate size. The sampling was undertaken by the standard Coning and Quartering technique and the final sample was prepared by pulverizing in the Teema Mill (Model, Jean Wirtz Dussel Dors, UK) for chemical evaluation and extraction studies.

Chemical evaluation. The sample was treated with an appropriate concentration of hydrochloric and hydrofluoric acids were heated on the sand bath. After cooling, water was added to it. The solution was filtered and its volume was measured in the measuring flask. Molybdenum and cobalt were estimated spectrometrically (Skoog and West 1971) using Atomic Absorption Spectrometer Model Hitachi, Z-8000. (Table 1), while alumina was evaluated using conventional method (Vogel 1961) by fusion with sodium carbonate.

Leaching/extraction studies. The catalyst was pulverized and then different techniques were applied for the leaching of molybdenum, cobalt and alumina on the sample. The conditions were optimized for the extraction of their respective salts. Following studies were undertaken on the samples.

(a) Acid leaching (b) Ammonia leaching (c) Soda leaching.

The brief description of the studies is given below:

a) Acid leaching: Different acids were tried for leaching by heating the sample with their different concentrations. The
SYNTHESIS OF SINENSETIN, A NATURALLY OCCURRING POLYMETHOXYFLAVONE

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5, 6, 7, 3’, 4’-Pentamethoxyflavone (8) isolated from the leaves of Orthosiphon stamineus has been synthesized by following an unambiguous route. All the new products have been characterised on the basis of spectral data and microanalysis.

Key words: Synthesis, Characterisation, Chalcone, Flavone.

Introduction

Flavonoids constitute an important group of natural products and some of them possess a wide range of biological activities such as antibacterial (Conn 1981), antifungal (Ghosal and Chaudhuri 1975), anti-inflammatory, antimicrobial, anti-tumour anti-cancer, prostaglandin binding (Conn 1981) and insect antifeedant (Ghosal and Chaudhuri 1975). Kielland et al. reported the isolation of 5,6,7, 3’,4’-pentamethoxyflavone (8), from the leaves of Orthosiphon stamineus. The structure for sinensetin was assigned on the basis of spectral data and synthesis (Matsuura et al. 1973). This paper describe a new synthesis of sinensetin (8) (Scheme I) in better yield (59%).

2,4,5,6-Tetra (methoxymethoxy) acetophenone (2). To a solution of 2, 4, 5, 6-tetrahydroxyacetophenone (1), (5g) in dry acetone (75 ml) were added methoxy methyl chloride (2 g) and anhyd. K₂CO₃ (40 g). The mixture was refluxed for 3 h. Acetone was removed by distillation and water was added to the residue. It was extracted with ether, dried over anhyd. Na₂SO₄ and evaporated to dryness. The ether extract on column chromatography using petrol (40-60 °C), petrol-benzene (4:1), petrol-benzene (4:3) and increasing quantities of benzene as eluents gave the major compounds (2) and several other minor compounds. It was oily liquid (3.99 g), (M + , 360); Rf 0.69 (benzene-acetone; 25:1); UV: 230, 245, 278 nm; IR: 2855, 2479, 1645, 1605, 1599, 1543, 1469, 1410, 1375, 1345, 1234, 1212, 1190, 1156, 1132, 1050, 1043, 1005, 945, 885, 765, 664 cm⁻¹; 1H-NMR: 2.48 (s, 3H, -COC₂H₃), 3.42 (s, 12H, -CH₂OC₂H₃ x4), 5.53 (s, 8H, -CH₂OCH₃ x4), 6.41 (s, 1H, H-3).

3,4-Di(methoxymethoxy)benzaldehyde (4). A mixture of 3, 4-dihydroxybenzaldehyde (3) and methoxymethyl chloride (2 g) and anhyd. K₂CO₃ (40 g). The mixture was refluxed for 3 h. Acetone was removed by distillation and water was added to the residue. It was extracted with ether, dried over anhyd. Na₂SO₄ and evaporated to dryness. The ether extract on column chromatography using petrol (40-60 °C), petrol-benzene (4:1), petrol-benzene (4:3) and increasing quantities of benzene as eluents gave the major compounds (2) and several other minor compounds. It was oily liquid (3.99 g), (M + , 360); Rf 0.69 (benzene-acetone; 25:1); UV: 230, 245, 278 nm; IR: 2855, 2479, 1645, 1605, 1599, 1543, 1469, 1410, 1375, 1345, 1234, 1212, 1190, 1156, 1132, 1050, 1043, 1005, 945, 885, 765, 664 cm⁻¹; 1H-NMR: 2.48 (s, 3H, -COCH₃), 3.42 (s, 12H, -CH₂OCH₃ x4), 5.53 (s, 8H, -CH₂OCH₃ x4), 6.41 (s, 1H, H-3).

3,4-Di(methoxymethoxy)benzaldehyde (4). A mixture of 3, 4-dihydroxybenzaldehyde (3, 2.8 g) in dry acetone (40 ml), methoxymethyl chloride (3.20 g) and anhydrous potassium carbonate (10 g) was refluxed for 15 min. The reaction mixture was worked-up as above and the solid obtained was crystallized from petroleum ether was white crystals (1.98 g), m.p. 54°C (Hossain and Islam 1993, m.p. 54°C).

Experimental

Melting points were determined using an electrothermal melting point apparatus (Gallenkamp). IR spectra were recorded (KBr discs) on a FT-IR spectrophotometer, valid...
Introduction

The discovery that some microorganisms could be used in modifying the steroid nucleus gave some lights about the field of steroid transformation (Petresson and Murry 1952). The most important reactions in the field of steroid transformation are the hydroxylation reactions. A large number of fungi have the ability to hydroxylate steroids in various positions. The major industrial interest has been focused on those capable of hydroxylating steroid at the 11 position, because it is the key step in cortisone and prednisone production.

The biological hydroxylation of steroids at this strategic position is by far the most important, where the presence of an oxygen function at C\textsubscript{11} is necessary for the anti-inflammatory activity of these compounds. This type of biological oxidation of steroids was not only confined to the enzymatic systems of microorganisms but also found in the mammalian tissue enzymes (Hanch et al 1949; Morfin 2000; Soffer et al 1961).

Aspergillus ochraceus NRRL 405 was used to hydroxylate progesterone to 11\(\alpha\)-hydroxyprogesterone (11\(\alpha\)-HP). This study described the effect of some fermentation parameters and the intermittent addition of \(\beta\)-cyclodextrin on the biotransformation process. The Kinaway’s medium with pH 6 produced the best result of the used culture media. The transformation period was 48 h for the maximum hydroxylation. The maximum production of 11\(\alpha\)-HP (93.10\%) was obtained by the addition of 4g/l \(\beta\)-cyclodextrin at 12 h after inoculation compared to the control culture (56.8\%). The results also showed the ability of the mould culture to carry out the transformation reaction at high substrate levels without by-products formation in the presence of \(\beta\)-cyclodextrin.

Key words: 11\(\alpha\)-Hydroxyprogesterone (11\(\alpha\)-HP), Residual progesterone (RP), \(\beta\)-cyclodextrin.
Integrated Biological and Chemical Treatment of Brewery Sludge

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Composite samples of sludges obtained from a brewery in Benin City were analysed for their pollution characteristics. The samples were then treated by integrated biological and chemical methods. The analysis revealed that the BOD and COD of the sludge liquor were high, as well as the levels of solids concentration, nitrogen, phosphorus and total bacterial counts. These showed that sludge from the brewery have high pollution potentials and therefore, needed treatment before disposal or reuse. The sludges were treated by integrated biological and chemical methods. Percentage solids reduction achieved were in the range of 21.4 - 27% total solid (TS), 27 - 32% suspended solids (SS) and 32 - 48% volatile solids (VS) for integrated aerobic/chemical treatment and 24.3 - 27.2% TS, 30.3 - 33% SS and 34.4 - 36% VS for anaerobic/chemical treatment. BOD and COD reductions were in the range of 97 - 98 and 97.3 - 98.2%, respectively for aerobic/chemical treatment and 97.7 - 97.9 and 98 - 98.3%, respectively for anaerobic/chemical treatment. Phosphorus, ammonia and nitrate nitrogen were found to be substantially reduced in this sludge thus preventing the eutrophication of water bodies, up to 57 - 85.2% NH₃ and 31.7 - 61.9% NO₃. Reduction was achieved using aerobic/chemical treatment while the anaerobic/chemical method achieved a reduction of up 100% NO₃.

Key words: Brewery sludge, Pollution, Chemical treatment, Biological techniques, Eutrophication.

Introduction

In the recent times, efforts have been geared towards the treatment of domestic and industrial wastewaters while sludges associated with them are merely dumped untreated into the environment. Many of the steps, taken to treat wastewaters results in the concentration of pollutants into a sludge (Priestly 1991), sludge can therefore, become unstable, putrescible and pathogenic. Thus sludges must be treated before disposal or reuse in order to alleviate pollution problems and create a good and healthy environment.

Sludge treatment processes can be categorized essentially into two generic types, one based on biological and the other on chemical techniques.

In biological systems of treatment, treatment principles involve oxidation of all biodegradable organics by bacterial action. Complex biodegradable substances are broken down into less complex ones to produce cleaner and clearer effluent. However, non biodegradable substances pass unaffected. In cases where there are heavy toxic metals or toxic substances in any wastewater or sludge to be treated, the lives of the bacteria to effect biodegradation of the waste may be killed and so little or no treatment occurs in the wastewater. Additionally, nitrates and phosphate present in any wastewater or sludge are not well reduced by any biological method; these substances are known to cause the problem of eutrophication in water bodies (Ademoroti 1996a). Nitrate has been found to increase in trickling filtration and surface aerated activated sludge treatment in tropical countries (Ademoroti 1983). Nitrate has also been found to increase in wastewater sludge treated by aerobic digestion due to nitrification (Asia 2000). Nitrate in water is known to cause the disease called methemoglobinemia in infants. Furthermore, reduction of metals is limited and toxicants are not reduced. All these are the demerits of biological methods of treatment.

Chemical method of treatment is to some extent applied to the treatment of sludge but it has many shortfalls as well. The quantity of sludge resulting after treatment is always sizeable especially when lime is used, and only a small quantity of ammonia is removed. In some studies conducted in Nigeria, only 5 - 8% ammonia was removed when alum and iron (III) chloride salts were used in wastewater treatment (Ademoroti 1982). This reduction is insignificant when 2-3 mg/l ammonia is known to have lethal effect on fish.

In this work, wastewater sludge from the brewery industry was characterized and treated by the integration of the biological and the chemical methods (aerobic/chemical and anaerobic/chemical methods). This was to ensure proper and effective treatment. This was considered adequate to produce a superior effluent and sludge to those of either method in isolation so that what is lacking in the biological methods is obtainable in the chemical method and vice versa.

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Introduction
Substances obtained from culture filtrate and cell wall of plant pathogen i.e. fungi elicit hypersensitive defence responses when applied to tissues or cell cultures of incompatible plants (Knogge 1996; Paiva 2000). These substances are chemically diverse in nature such as polysaccharides (Clarence and Edward 1991), proteins and glycoproteins (Voglsang et al 1994), glycolipid/lipids (Castoria et al 1995). Elicitor active material from Colletotrichum lindemuthianum, the causal agent of anthracnose disease in beans is first known from culture filtrate of hot water extracts of fungal cell wall (α race) (Anderson and Albersheim 1975), the active material shown to contain a high molecular weight β-1, 3 and 1,4-linked glucose polysaccharides. Subsequently, elicitor active preparations were studied from culture filtrate of the α (Anderson 1978; Anderson 1980a), β (Anderson 1980b, Tepper and Anderson 1986) and IMI 112166 (Hamdan and Dixon 1986, 1987) races. The accumulation of isoflavonoid phytoalexins was studied in several Colombian bean cultivars resistant and susceptible to C. lindemuthianum (Dieg et al 2002).

In these studies, methanolysis and hexane extraction of partially purified glycoconjugate obtained from culture filtrate of C. lindemuthianum IMI 112166 provided a series of fatty acids on separation and characterization by GC and GC-MS.

Materials and Methods
Elicitor preparation and purification. Shake cultures of Colletotrichum lindemuthianum, the causative agent of anthracnose in beans (Phaseolus vulgaris) was successfully cultured in a complex medium of glucose/neopeptone. The extracellular High Molecular Weight Culture Filtrate Elicitor (HMWCFE) was isolated by simultaneous dialysis and ultrafiltration of nominal cutoff 30,000 dalton cartridge systems. Chromatographic separation provided two fractions Partially Purified Fraction, PPF-I (Mr 2000,000) and PPF-II (Mr < 40,000). Extraction with organic solvents showed that lipid was found in crude preparations. Methanolysis of PPF-I and II analyzed by GC, provided a series of fatty acids characterized by GC-MS viz: behenic (C22), arachidic (C20), isostearic (C18), oleic (C18:1), palmitic (C16), myristic (C14) and capric (C10) acids. Not all the lipid material was released under mild basic hydrolyzing conditions indicating the presence of some stable linkages between lipid and sugar moieties.

Key words: Colletotrichum lindemuthianum, Elicitor, Fatty acids, Carbohydrate.
Influence of Common Purslane Aqueous Extracts on Germination and Seedling Growth of Rice

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Aqueous extracts of various parts of leaf, stem and root of common purslane were evaluated alone or in combination with NaCl to see their effects on germination and seedlings growth of rice. Leaf and stem extracts have no effects on germination, while the root extract along with 0.4% NaCl decreased the rice seed germination. However, length of both shoot and root were significantly decreased under extracts alone or in combination with NaCl levels. Root growth was affected more than the shoot irrespective of the treatments.

Key words: Common purslane, Aqueous extracts, Rice.

Introduction

Common purslane (Portulaca oleracea L.) is an annual and very common weed in warm areas (Horowitz 1971). The common purslane, locally known as kulfa or lunak is commonly found in maize and berseem fields as well as along the sides of canals. The allelopathic effects of this weed on crop growth have been reported in a number of studies (Alam et al 1990, 1997, 1998, 2001a; Anaya et al 1987).

The aqueous extract of common purslane inhibited the seed germination, coleoptile and root growth of Mida wheat (Triticum aestivum) in an earlier investigation (Le Tourneau et al 1956). They reported that pH and osmotic potential of the extract was not responsible for reduction. Gressel and Holm (1969) found that weed seeds of purslane reduce the seed germination and growth of alfalfa and radish due to release of allelochemicals. Pope et al (1984) found that root exudate of common purslane significantly reduced the seedling growth of soybean, root growth of radish and tomato. Using different weed’s parts on the growth of wheat, it was found that germination, shoot and root lengths significantly reduced with aqueous leaf, stem and root extracts of scarlet pimpernal and bermuda grass (Alam et al 2001b, 2001c).

Using several weed parts, in a short term experiment, Alam (1996) has reported that parts of weed are; common lambsquater, bermuda grass, purple nutsedge, common purslane, field bindweed etc have reduced the germination and seedling growth of wheat and rice. Anaya et al (1987) reported that leachate of fresh common purslane produced high inhibition of radicle growth of corn, bean and squash. It was observed that shoot and root leachates of common purslane caused the greatest reduction in seed germination. Shoot leachate caused greater reduction in seedling growth as compared with root leachates. Phenolics such as coumorin benzoic and ferulic acid were found in the extract (Dharmaraj et al 1988). In a bioassay test common purslane showed an allelopathic potential against clover (Trifolium repens) and lettuce (Souto et al 1990). This study was therefore, carried out to evaluate the effects of different parts of common purslane on the seed germination and seedling growth of rice.

Materials and Methods

The common purslane plants were collected, washed with distilled water and dried in an oven at 70°C for 24 h. Each plant was separated into leaf, stem and root. They were dried in the oven at 70°C for 48 h. The dried samples were ground in a Wiley mill to pass through a 20 mesh screen. The aqueous extracts of leaf, stem, root were prepared by soaking 5 g of powdered materials of each part separately in 100 ml of distilled water for 24 h. The extracts were filtered using whatman filter paper no. 42 and kept in reagent bottles. Five ml of the filtered aqueous extracts each of leaf, stem and root from the filtered solution were added separately to 0.08% agar gel supplemented with the levels of 0.0, 0.2 and 0.4% NaCl. Fifty ml of the agar media of each treatment was poured into a series of glass bowls. A similar set, but without leaf, stem and root extracts were prepared to determine the NaCl affect alone, while the bowls with only 0.8% agar were considered as control. Rice seeds of Shua-92 were surface
LIPID CLASSES OF *HORDEUM VULGARE*

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Two local varieties of *Hordeum vulgare* Jao 87, and Jao 83 were studied for their lipid classes and fatty acid composition. Total barley lipids were solvent extracted and classified into their respective classes with the help of silicic acid gel column chromatography. Each lipid class was further fractionated by thin layer chromatography, and after methylation, its fatty acid composition was determined by gas chromatography. The lipid contents were 3-3.2% of the total barley weight in both the varieties. The percentages of neutral lipids, glycolipids and phospholipids, were 70, 10 and 20%, respectively. Gas chromatography showed the presence of fatty acids from series lauric (C12:0) to arachidic (C20:0) while linoleic acid (C18:2) was the principal fatty acid in all the classes with a few exceptions.

**Key word:** Hordeum vulgare, Lipids, Fatty acids, Methyl esters, GC, TLC.

**Introduction**

Barley, *Hordeum vulgare*, of the family gramineae, (locally identified as Jao) is one of the oldest cultivated cereals. Barley is well-known for its nutritious and medicinal properties. It has been used as food by human beings and fodder for animals. It is also extensively used in pearlizing, malting and brewing.

*Hordeum vulgare* is six-rowed type barley variety, cultivated in rainfed and irrigated areas of Punjab. About 1,40,000 tonnes of barley per year is produced in Pakistan. Barley matures quicker than wheat and can be cultivated in climatic conditions which do not permit the survival of wheat. The caloric value of barley is lower than that of wheat and corn, the most efficient method of increasing this value with least disturbances in properties is to increase the lipid content, which can be achieved by growing healthier barley grains. Agriculture Research Institute, Faisalabad, has developed some barley varieties which give healthier barley grains and produce more crop/acre. These are well-adjusted to the local climatic conditions, absorb the fertilizer easily and have better resistance for diseases.

Most of the studies on barley concern distribution of lipids and the fatty acid composition in ripening and maturing grains (De Man and Caubergh 1988), changes in lipid quality during malting and brewing processes (Narziss and Mueck 1986; De Varies 1990) identification of phospholipids of barley (Aylward and Showler 1962), identification of neutral and polar lipids (Parsons and Price 1974; Price and Parsons 1974) and effect of barley diet on lipid metabolism and diet digestibility in model animals (De Peters and Tayler 1985; Wang et al 1993).

The present work was undertaken with an idea to study the lipids of barley varieties cultivated in Pakistan. The improved barley varieties Jao 83 & Jao 87 were collected from Agriculture Research Institute, Faisalabad, and evaluated for their individual lipid classes, i.e neutral lipids, glycolipids and phospholipids to obtain detailed information about the fractions of each of these classes.

**Materials and Methods**

*Extraction of lipids.* The dried whole grain sample of barley (*Hordeum vulgare*) was crushed in an iron pestle and morter and then converted to a uniform powder with an electric grinder. The extraction of lipids was undertaken immediately to minimize any oxidative and enzymatic activity. The ground sample (50g) was placed in 1 litre beaker and stirred with 10 volumes of chloroform: methanol: water (1.0:1.0:0.9 v/v), with a magnetic stirrer for ½ an hour, using the method of Price and Parsons (Price and Parsons 1974). The mixture was transferred to 1 litre separatory funnel, swirled for further solvent action, stirred with a glass rod to enhance solvent layering and allowed to stand overnight. The lipid charged chloroform layer was then collected in a round-bottomed flask, and restored to the original volume by chloroform. This was repeated twice. The pooled lipid extracts were evaporated to dry-
**Dispersing and Parasitizing Ability in *Trichogramma chilonis* Ishii in Early and Late Sown NIAB-86 Cotton Variety**

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Egg parasitoids *Trichogramma chilonis* were released in cotton by planting after early and late sown NIAB-86 to find out dispersing and parasitizing potential. Observations on percent parasitization were taken at distances of 1-5 meters from fixed releasing sites. It was recorded that the dispersal of *T. chilonis* was physically affected by crop growth and climatic conditions. The maximum dispersal and parasitization was recorded in late September and in early October. Intensity of parasitization was more after 24 h as compared to 48 h post release of parasitoids.

**Key words**: Dispersal, *Trichogramma chilonis*, Parasitization, Cotton.

**Introduction**

Egg parasitoids, *Trichogramma* spp. have been proved very beneficial as biological control agent in cotton crop (Ahmad *et al* 1998). Their releases in many crops are being made since the past 25 years. However, trials with these parasitoids prior to 1975 were aimed to control lepidopterous pests only in sugarcane and corn crops. Afterwards, from 1975 to 1985 these were applied for the control of cotton, cabbage, apple and tomato’s pests etc. (King *et al* 1986). Currently, their inundative releases are being practiced in more than 50 countries and on an area of more than 32 million hectares to control lepidopterous pests every year around the world (Hassan 1993). Use of *Trichogramma* spp. is therefore, considered an effective and alternative mean of pest control, while comparing with other traditional approaches of pest control (Rehman *et al* 2003).

Dispersal ability of an insect is a fundamental element in its life history and ecology, is often judged by its range of flight. Dispersal can be hindered by individual itself and environmental factors that often interact together (Lidicker and Stenseth 1992). Parasitoids prove more effective with their dispersal and searching parasitizing ability is well adapted in the field. It was observed that the dispersal of *T. chilonis* is affected by the changes in the environmental conditions (Biever 1972; Lewis *et al* 1976).

The objective of the present studies was to find out the performance of *T. chilonis* in respect of its dispersal and parasitizing potential on a commercial cotton variety NIAB-86 sown on two different times.

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**Materials and Methods**

A commercial cotton variety NIAB-86 was planted on two different sowing times, i.e. early and late with fifteen days interval. Fertilizer application to crop; irrigation and all other agronomic practices were same as per crop requirement in both plots i.e. in early and late sown conditions. Three replications were made for each sowing time under Randomized Complete Block Design (RCBD). The *T. chilonis* strain used in the present experiment was collected from the parasitized eggs of *Helicoverpa armigera* (Lepidoptera; Noctuidae) on cotton crop in 2000 at entomological field area of NIAB, Faisalabad. Parasitoids have been reared in biological control laboratories of NIAB at 25 ± 2 °C, 75 ± 5% relative humidity for almost 70 generations on *S. cerealella* (Lepidoptera: Gelechiidae) eggs. Five Tricho-cards, each having three thousands *T. chilonis* adults ready to emerge from host eggs and they were installed at five different locations in cotton field. Blank unparasitized *S. cerealella* host eggs, pasted on cards, were exposed to Tricho-cards at the distance of 1, 2, 3, 4 and 5 meters by using single parasitoid card to a single blank card for respective distances. Three repeats were made to each set of treatment covering all distances. The host cards were collected after every 24 and 48 h after post installation of Tricho-cards. The released parasitoid cards were checked under stereo binocular microscope to determine percent parasitization of eggs by the parasitoids that have traveled the distance in the field. Data was recorded in cotton field on weekly basis from July to October 2000 interpreted by using DMR-test to differentiate treatment means (Steel and Torrie 1984).
Reclamation of Waste Lubricating Oils Using Ground Periwinkle Shell Adsorbents

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Acid treated ground periwinkle (Fuscutus tympanotonu) shell adsorbents were used in the reclamation of used hydraulic oil by both the continuous elution and bed filtration techniques. Quantitative yields of oils were obtained by both techniques. The bed filtration technique gave clear oils but was slower than the continuous elution technique. Comparison with conventional adsorbents showed that the ground shell adsorbent was comparable to fuller's earth and better than silica or alumina. Elevated temperature oxidation of the reclaimed oils in the presence of atmospheric oxygen gave resins, which could be used in the production of paints, leather polishes and wood varnishes.

Key words. Lubricating oils, Ground periwinkle shell, Fuscutus tympanotonu.

Introduction

Handling and disposal of used lubricating oils are increasingly becoming a matter of concern to environmentalists, governments, industries and research scientists (Swain 1978). Indiscriminate disposal of used oils on the soil around automobile maintenance garages and into waste water streams by many industries constitute serious pollution hazards to both terrestrial and aquatic environments (Nemerow 1978; CONCAWE 1987). Also burning of used oils as a means of disposal is identified as a source of atmospheric pollution through widely dispersed distribution of heavy metal oxides and damaging gases (Becker 1982). Besides the pollution hazards, these methods of disposal of used oils are considered a waste of valuable resources as most used oils still contain substantial amounts of recoverable base oil.

Therefore, taking into account the stringent environmental regulations leading to higher cost of disposal coupled with the generally rising costs of petroleum products and recycling of used oils is now considered a viable option for a non-polluting disposal and at the same time conservation of oils (Swain 1978). Various processes available for used oil reclamation are reported in comprehensive reviews by Cotton (1979) and Brinkman et al (1987). Of the available reclamation processes, adsorption purification using various adsorbents such as bleaching clay, bauxite, silica gel, activated carbon, char bone, etc is the oldest and had remained one of the most effective and cheapest methods. An earlier study (Ofunne et al 1989) showed that the use of the naturally available, easily purified and regenerable sharp sand was as effective as fullers earth as an adsorbent for the reclamation of used hydraulic oil.

In the present study, the effectiveness of another readily available material as an adsorbent for used hydraulic oil reclamation is investigated. The material, periwinkle (Fuscutus tympanotonu) shell is a food process waste and litters most of Nigeria’s coastal towns. Chemical compositional analyses showed that it contains mainly calcium carbonate and silicates which are similar to the constituents of some of the well-known adsorbents such as the bentonites and florisil. Thus the investigation of its potentials as an adsorbent was thought worthwhile technically and as an environmental sanitation measure.

Practically oil can be reemployed in its former service once its equivalency with the original base oil has been established and necessary reformulations are made (Jacobs 1977). However, the engine tests required to establish the equivalency of a reclaimed oil with the original base oil that are usually very expensive. Moreover, refining is not always feasible because highly specialized and expensive facilities are involved. Therefore, it was sought to find other uses for the reclaimed oils in the study, by converting to resins, which could be useful to other industries.

Experimental

Materials. The used mineral based hydraulic oil was obtained from the D.R. shaft hydraulic systems at the Delta Steel Company Limited, Aladja, Nigeria. Chromatographic alumina and silica gel (70 - 230 mesh) were products of Merck. The periwinkle shells were obtained from the roadsides in Port Harcourt. N-Hexane (BDH) was the eluting solvent.

Treatment of the periwinkle shell. The shells were washed, dried, ground and sieved with a 500 µm mesh sieve.
The comparative production performance and the feasibility of production of fish in unutilized seasonal mini ponds under farmer’s condition through culturing silver barb (*Barbodes gonionotus* Bleeker) in mono and mixed culture with Genetically Improved Farmed Tilapia (GIFT) (*Oreochromis niloticus* L) was investigated in six seasonal mini ponds of 0.02 ha each for five months. Silver barb mono culture (Treatment-1) and mixed culture (Treatment-2) with GIFT were tested with stocking density of 16,000/ha of fish for both treatments. There was no significant variation on either water quality parameters or abundance of planktonic organisms due to different culture systems of silver barb. In mixed culture (T2) system, GIFT ranked 1st position in the production (1442.90 kg/ha) and the individual production of silver barb was 856.36 kg/ha. A significantly (P < 0.05) higher total production (2299.26 kg/ha) of fish and net benefit (58,383.12 TK/ha or US$ 1004/ha) was recorded in the mixed culture (T2) than that of the total production (1606.53 kg/ha) and net benefit (31,774.26 TK/ha or US$ 546.42/ha) of monoculture system (T1).

**Key words:** *Barbodes gonionotus*, *Oreochromis niloticus* L. Mono and mixed culture, Mini ponds, Farming system.
**Introduction**

Phytic acid is myo-inositol-1, 2, 3, 4, 5, 6-hexakis-dihydrogen phosphate. In most seeds it serves as primary phosphorous and myo-inositol reserve. It also stores other cations and energy (Cosgrove 1966). Binding of phytic acid with minerals is pH dependent; and complexes with different cations have varying solubilities. Phytic acid also binds protein molecules (Shwenke et al. 1986; Mothes et al. 1987) leading to decreased solubility, functionality and digestibility of proteins (Maga 1982).

Binding of minerals with phytic acid also results in their reduced physiological availability (Cosgrove 1966). It is known to decrease the bioavailability of Ca, Fe, P, Zn and other trace elements to humans and mono-gastric animals. This leads to severe nutritional and consequently health problems in the consuming populations (Smith and Circle 1978; Maga 1982; Thompson 1987). Iron deficiency anemia (IDA) is a severe problem of public health significance in Pakistan. Quite a large proportion of various population groups are affected (Anon 1988), resulting in low Hb levels, reduced working and mental capabilities and ultimately in great economic losses (Joseph 2000). Phytic acid is considered to be one of the most significant confounder in this connection. Logically any R & D endeavor to improve the micronutrient nutriture of the population will include a component on phytic acid research.

Several methods have been reported for the determination of phytic acid. However, a rapid and sensitive method, suitable for screening large numbers of samples, is still required. In the conventional method (McCance and Widowson 1935) the phytic acid is precipitated as ferric phytate. The precipitate is separated, washed, and the phosphorous content determined after hydrolysis. The phytic acid content is calculated from the phosphorus content. This method is very time consuming.

A relatively faster method was developed by Haug and Lantzsch (1983), by modifying an indirect method originally described by Young (1936). The sample extract is heated with an acidic Iron III solution of known iron content. The decrease in iron (determined colorimetrically with 2,2-bipyridine) in the supernatant is a measure for the phytic acid content. This method has several shortcomings at the practical level and we had to face problems of reproducibility of results while using that procedure. Color developed with pyridine reagent fades down quickly and hence the time of taking absorbance (OD) influences the results. Line drawn to plot the concentration against OD becomes parallel to X-axis beyond 30 ppm concentration, indicating in sensitivity of the procedure in that range. Apart from using expensive reagent (2,2-bipyridine), the method is not recommendable for large number of samples. A need therefore was felt for developing a method with high degree of reproducibility and sensitivity in larger concentration ranges. Since iron can be determined with