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Detection of Compounds of Mercury, Cadmium and Copper by Baker's Dry Yeast Enzyme Inhibition

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Abstract: Mercuric chloride compound can be detected by Baker's dry yeast succinate dehydrogenase enzyme as sensor on micro TLC plate and on Whatman No.3 filter paper at 0.5 μg amounts as white chromatograms. Copper sulphate and cadmium sulphate compounds can also be detected at 1.0 μg amount. The Baker's dry yeast enzyme inhibition method is reported for the first time on mirco TLC plate and on Whatman No.3 filter paper which is rapid, sensitive and a low cost method for large scale screening of mercuric compound qualitatively in market or field or small laboratories before it is being taken to Federal or Central Laboratories for determination by sophisticated and costly equipments. Mercuric chloride, cadmium and copper compounds can also be detected, separated by Biochromatographic method using 0.1% NaCl as solvent system with Baker's dry yeast succinate dehydrogenase inhibition method employing INT:sodium succinate:PMS chromgenic reagent. The method is cost effective with easily procurable potential biosensor for detection of microgram amounts of compounds of mercury, cadmium and copper in laboratory or field on micro TLC plate or on Whatman No.3 filter paper as white inhibition spots or white chromatograms.

Key words: Biodetection of Mercuric compound · Baker's dry yeast sensor · $CdSo_4$, CuSo Biodetection · Succinate dehydrogenase sensor · Bio micro TLC and Paper Chromatography · Low Cost Device · Developing countries

INTRODUCTION

During the last three decades there has been a rapid industrialization for enhancing the living conditions, standards and economic advancement of mankind. As the size of the world's population becoming larger, the uses of materials and energy show parallel increases and the rivers and oceans are at receiving end to accept some of the wastes of society. Toxic metals such as mercury, cadmium, arsenic, copper and many other heavy metals tend to accumulate in bottom sediments from which they may be released by various processes of remobilization and biomagnifications and move up the food and biologic chain thereby reaching human beings causing chronic and acute ailments [1].

Industrial pollutants are more difficult to characterize and detailed inventories of industrial wastes on a national level in developing countries are yet lacking [2]. There are a variety of industries which contribute to heavy metal pollution in the environment [3]. Khalil [4] described the impact of water pollution which contained mercury on productivity and fisheries of Lake Mariut in Egypt and the polluted water showed much higher concentrations of some heavy metals of which the mercury played a major role. Khalil [4] reported that mercury amounts ranged from 0.01 to 0.02 mg per liter. The mercury was also detected in liver of fish. Khalil [4] reported that the fish production in Lake Mariut began to decrease shortly due to the enormous increase of industrial wastes and sewage of Alexandria discharged into the lake. Though Absorption spectroscopy [5], Neutron Activation Analysis [6], Anode Stripping Voltametry [7], Colorimetry, Potentiometry [8,9], Emission Spectroscopy [10] are ideal for accurate determination, but they are sophisticated, cumbersome, costly and not affordable in developing countries. In the present research paper a low cost method was described for preliminary large scale qualitative screening with "Bakers Dry Yeast" sensor

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before being taken to Central Federal Laboratories. Samples which showed heavy metal positive reaction can only be analysed for cost effective approach. From the point of procurability sensitivity portability and quick detection of mercuric chloride compound the present 'Bakers dry yeast sensor system' method is superior than earlier methods reported by Nanda Kumar and Prameela Devi [11], Prameela Devi and Nanda Kumar [12] with mammalian liver freeze dried extracts for detecting a variety of heavy metals [12] or chick brain for lead compounds [13,14]. Biological sources are not only sensitive but specific compared to chemical methods of detection [15, 16] of various pollutants.

In view of above methods of detection of heavy metals it is felt necessary to develop a new detection method employing a biological source which is a low cost and easily procurable but potentially sensitive sensor namely Baker's yeast dehydrogenase. The biosensor detector system is a new development and the present investigation describes the method developed. The method is also applicable for detection of copper and cadmium compounds in laboratory or field.

MATERIALS AND METHODS

Reagents: (a) Mercuric chloride: 97% pure, S.D. Fine Chemicals, Mumbai. (b)Copper sulphate and cadmium sulphate: 98% pure, S.D. Fine Chemicals, Mumbai. (c) Silica gel G: LOBA-Chemie Indo-Austranal Co. Mumbai. (d) Sodium chloride: LOBA-Chemie Indo-Austranal Co. Mumbai. (e) Sodium succinate: LOBA-Chemie Indo-Austranal Co. Mumbai. (f) INT (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5 Phenyl tetrazolium chloride: LOBA-Chemie Indo-Austranal Co. Mumbai. (g) PMS (N-methyl phenazonium methosulphate): LOBA-Chemie Indo-Austranal Co. Mumbai. (h) Mercuric chloride aqueous standards: 10.00 mg Mercuric chloride was dissolved in 100 ml distilled water and was used for detection on silica gel plate. (i) 10.00 mg Mercuric chloride was dissolved in 100 ml fresh water from a pond. (j) 10 mg Copper sulphate, (k) 10 mg Cadmium sulphate was dissolved separately in 100 ml fresh water from a pond.

Grapes sprayed with heavy metals and extracted using sodium citrate, neutralization with buffer, concentration by evaporation/precipitation with alkaline buffer as described by Prameeladevi and Nanda Kumar [12].

Enzyme Preparation: "Baker's Dry Yeast" in granule form was procured from market and 10% W/V homogenate was prepared in distilled water by grinding in mortar with pestle or an electoral homogenizer at room temperatures (28-38°C). The homogenate was used as Succinate dehydrogenase enzyme sensor source for detection on silica gel plate. In temperate countries (cold countries) homogenate can be prepared in warm (25-40° C) water just before use. Dry yeast granules in air tight containers have long shelf life and is portable.

INT: Sodium Succinate Substrate: Pms Chromogenic Reagent: 0.4% (W/V) INT was prepared in distilled water and stirred with glass rod for proper dissolution. Sodium succinate was prepared in distilled water. 0.1% (W/V) of PMS was prepared in distilled water. The three reagents INT: Sodium succinate: PMS were mixed in volumes of10:10:2 ratios respectively.

Solvent System: 0.1% NaCl in distilled water.

Preparation of Silica Gel Coated Glass Plates: 7.5x2.5 cm clean glass slides were coated with layer of silica gel G in water slurry as described by Prameela Devi [12], Saritha [15] and Aparna [16]. Mirco TLC plates are used for separation, chromatography and also detection.

Preparation of What No. 3 Paper Strips: 7.5x2.5 cm Whatman No.3 filter paper strips are used in place of TLC plates [12] for paper chromatography and for detection.

Standard or Sample Application: With the help of 10 µL graduated micro capillary (with pointed edge) appropriate amounts of mercuric chloride standard or samples were spotted. Amounts ranging from 0.5 µg to 3 µg were applied on silica gel plate above the base edge or Whatman No.3 filter paper. Precaution was taken to minimize spreading of applied spot by frequent drying with hair drier or breeze.

Spot Detection: Baker's dry yeast homogenate (10% W/V) was sprayed on silica gel plate (micro slide) or Whatman No. 3 paper containing different concentrations of mercury from a chromatographic glass sprayer as a fine mist. The yeast enzyme solution should be deposited uniformly on the plate thoroughly wetting the gel or paper. The enzyme should not be sprayed in excess resulting in leaching on TLC plate or paper. The plates and Whatman No.3 filter papers were allowed to stand at room temperature for three minutes. They were removed and INT:PMS:Sodium succinate chromogenic mixture was sprayed uniformly as a fine mist without causing leaching

[12] for observing chromatograms. [*Alternatively for detection Whatman No. 3 filter paper (7.5 x 2.5 cm)* soaked in dry yeast homogenate can be placed on filter paper spotted with heavy metal sample sandwiching between slides for uniform absorption of yeast homogenate. If wetting of homogenate is not uniform non-wetted areas show white color due to absence of homogenate. This procedure is adopted when file glass sprayers (50 ml volume) are not used. Paper chromatographic detection is ideal for large scale screening of spot detection of heavy metals and not for solvent separation and detection. This method is ideal for field detection on paper]. Now the paper or TLC plates were observed for appearance of white chromatogram against pink formazon background. About 5 minutes are required for proper appearance of chromatograms i.e white spots amidst pink formazon colour back ground. In field or market TLC plates can be warmed to 30-40°C with candle flame if ambient temperature is low. In case of paper, the paper can be placed on a micro slide and warmed lightly over a candle flame. Alternatively slide warmer instrument with 37°C can be used in laboratory. Similarly Copper and Cadmium compounds are detected. If the sample consists of above compounds at non-detectable limits the volume of application on plate can be increased. The white chromatograms are white enzyme inhibition spots.

Principle of Biochemical Reaction: Mercuric chloride or copper or cadmium compounds are used as fungicide. Mercuric chloride inhibits Baker's dry yeast succinate dehydrogenase. This biochemical reaction is operative on silica gel plate or on paper. The succinate dehydrogenase, a member of citric acid cycle present in yeast extract acts on sodium succinate substrate metabolizing sodium succinate into fumerate in a dehydrogenase reaction [17]. During this reaction the electrons are liberated which in turn reduce the tetrazolium salt to a pink coloured complex namely formazon [12] and quickly catalysed by PMS. The formation of farmazon (a reduction product) is due to acceptance of electrons after dehydrogenation occurring due to enzymatic reaction. In the presence of mercuric chloride, this enzymatic reaction is inhibited and white spots appear as inhibition spots. The type of inhibition is non-competitive inhibition. The inhibitory nature of these compounds was made use of as a valuable technique for detection on silica gel plate or on Whatman No.3 filter paper. This principle is operative for copper sulphate and cadmium sulphate compounds.

Mercuric Chloride Clean up and Extraction from Sample Water: A simple clean up procedure is adopted to suit enzymatic method of detections as reported by Prameela Devi and Nanda Kumar [12]. The mercuric chloride is extracted employing precipitation method or concentration by evaporation [12]. In the conventional chemical methods of extraction acids are used which would interfere with enzyme causing enzyme protein denaturation. Hence, in the present investigation for yeast "enzyme compatibility" concentration of heavy metals water sample was done by evaporation procedure [12] and was followed for mercuric compound or precipitation procedure for copper and cadmium compounds [12]. The sample which is evaporated to 1 ml from 500 ml or 1 litre helps in concentrations of mercuric chloride. If 1 mg is added in 1 litre and concentrated to 1 ml would yield approximately 1 μg per μL or 0.5 μL having 0.5 μg amount on micro TLC plate. The materials are portable and appropriate procedure described in Materials and Methods section can be adopted.

RESULTS AND DISCUSSION

Detection: Different concentrations of mercuric chloride in distilled water were spotted on a silica gel plate starting from 0.5 μg to 3 μg with help of a micro capillary from the extracts described above (Fig. 1). Caution was taken to see that the mercuric chloride spot do not widen. Application or spotting is done in small volumes so that the white chromatogram will not spread and widen on silica gel plate (Fig. 1). When higher amounts were spotted it is dried frequently to avoid widening of spot. The figure 1shows detection of mercuric chloride on silica gel plate at lower concentration the spot appears as feeble spot (serial number 1) and as the concentration increases (serial number 1, 2, 3) the spots appear as bright spots (Fig. 1). The mercuric chloride amount below 0.5 μgm did not show any white spot on silica gel plate. Hence it may be concluded that the minimum detectable limit for mercuric chloride by yeast extract succinate dehydrogenase inhibition was 0.5 μg amount (Fig. 1). The appearance of cadmium sulphate and copper sulphate at minimum detectable amount of 1.0 μg is given in Fig. 2 on TLC plate. Similar reaction is seen on Whatman No. 3 paper also.

The Figure 3 shows appearance of mercuric chloride after chromatographic separation on micro TLC plate with 0.1% NaCl as solvent system. The solvent 0.1% NaCl selected has no effect on enzymatic method of detection [12] as intracellular environment also consists of NaCl.

Fig. 1: Spot detection of different concentrations (0.5, 1 and 3 µg) of $HgCl_2$ on thin layer chromatographic plate by Baker's dry yeast SDH inhibition method.

Fig. 2: Spot detection of cadmium sulphate and copper sulphate on thin layer chromatographic plate by Baker's dry yeast SDH inhibition method at 1 µg amount.

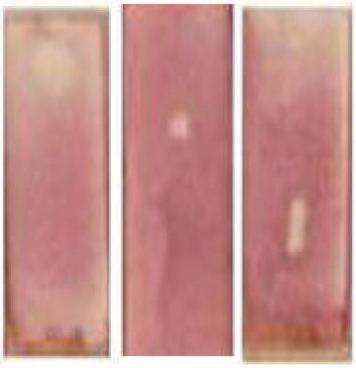

Fig. 3: Chromatographic separation and detection of mercuric chloride, cadmium sulphate and copper sulphate on TLC plate with 0.1% NaCl solvent system by Baker's dry yeast SDH inhibition method.

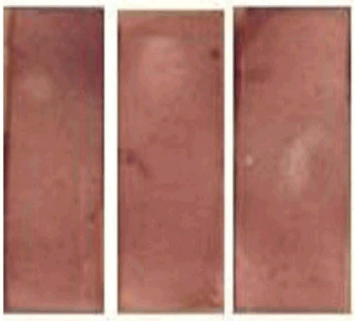

Fig. 4: Chromatographic separation and detection of mercuric chloride, cadmium sulphate and copper sulphate on Whatman No.3 filter paper strip with 0.1% NaCl solvent system employing Baker's dry yeast SDH inhibition method.

It is concluded that mercuric chloride compound can be detected employing Baker's dry yeast succinate dehydrogenase on a silica gel plate for detection at 0.5 µgm amounts. Mercuric chloride, Cadmium sulphate and Copper sulphate showed Rf value of 0.82, 0.48, 0.18 with 0.1% NaCl as solvent respectively (Fig. 3). The same procedure can be adopted for Whatman No.3 paper. Figure 4 shows appearance of $HgCl_2$, $CdSo_4$ and $CuSo_4$ on Whatman No.3 filter paper strips with Rf values of 0.87, 0.94 and 0.51 respectively (Table 1).

Interference by Other Compounds: Compounds such as strontium, magnesium, ferrous, manganese, calcium, chlorides, alluminium, organo phosphorous compounds, organo chlorides has no interference as they are not inhibitors of dry yeast enzyme system, an added advantage of biological method of analysis. For example organophosphate pesticides inhibit cholinesterase and not SDH on TLC plate [16] or on paper.

Merits: The detection method using yeast mitochondrial succinate dehydrogenase has not been reported. The method is simple low cost and does not require sophisticated analytical equipments. The time for analysis is much less, the method is less cumbersome. The specificity of inhibition by mercuric chloride on silica gel plate eliminates the interference by non-inhibitors. The Rf values are specific and for Copper and Cadmium compounds mixture showed different Rf values. The method employing Baker's dry yeast succinate dehydrogenase is more sensitive than the enzymatic methods of Prameela Devi and Nanda Kumar [12]. The Whatman no.3 paper method can be introduced as a practical method for Graduate classes without requiring costly equipments. The method can be employed for large scale screening in developing countries as it is simple to operate by non-skilled persons. The method can be applied for detecting fruit surface deposits sprayed with fungicides with heavy metals and thier washates in water.

Demerits: The method is suitable only for water samples, which is contaminated by metal compounds of mercury, copper and cadmium and heavy metal washates from samples. It requires simple clean up different from cumbersome chemical conventional methods where acids are used commonly for extraction. Simple clean up Method of Prameela Devi and Nanda Kumar [12] is adopted for enzyme campatibility. The method developed is a qualitative method useful for detection only and not for quantification.

Table 1: Detection of compounds of Mercury, Cadmium and Copper in fortified freshwater and surface residues of grape fruits

Fortified sample 10 mg/100ml	Paper Chromatography Rf value	MicroTLC Rf value	Solvent system
HgCl2 (grapes)*Pond water	0.87±0.09	0.82±0.071	0.1% NaCl
CdSo4 (grapes)*Pond water	0.94±0.08	0.48±0.024	0.1% NaCl
CuSo4 (grapes)* Pond water	0.51±0.043	0.18±0.006	0.1% NaCl

* Grapes sprayed with heavy metals and extracted using sodium citrate, neutralization with buffer, concentration by evaporation/precipitation with alkaline buffer as described by Prameeladevi and Nanda Kumar [12].

Values are mean of 6 observation

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