Xanthine (3, 7-dihydro-purine-2,6-dione) present in most human body tissues and fluids, is generated from guanine by guanine deaminase and from hypoxanthine by xanthine oxidase. Determination of xanthine level in blood and tissue is essential for diagnosis and medical management of various diseases like hyperuricemia, gout, xanthinuria and renal failure. A number of mild xanthine derived stimulants like caffeine and theobromine are present in tea and coffee. Fresh fish meat is required in food industries for manufacturing of high quality products. After the death of a fish, ATP is degraded into xanthine, which increases with storage. Thus xanthine attracts much attention as an indicator for fish freshness (Shan et al., 2009a). Various methods have been devised for determination of xanthine like enzymic colorimetric (Berti et al., 1988), HPLC (Kock et al., 1993), enzymatic fluorimetric, fluorometric mass spectrometry fragmentography (Olojoa et al., 2005) and capillary column gas chromatography (Renata et al., 2002). However, these methods are cumbersome, time consuming, requires sample preparation expensive apparatus and skilled person to operate. As compared to them, amperometric determination of xanthine by biosensors is comparatively simpler, rapid, sen-sitive and requires no sample preparation (Shah, 1996). In these biosensors, xanthine oxidase has been immobilized onto various supports such as theophylline coated nylon mesh (Moody et al., 1987; Mao et al., 1999), silk fibroin membrane (Mulchandani et al., 1989), self-assembled phospholipids membrane (Rehak et al., 1994), silk membrane (Mao et al., 2001), nafion membrane (Nakatani et al., 2005), cellulose acetate membrane (Basu et al., 2005), polypyrrole film (Arslan et al., 2006) and PVC membrane (Pundir et al., 2012). However these supports (film/membranes) had few drawbacks such as poor stability, reusability and slow electron transfer, while some membranes were fragile, non-conducting, non-elastic and had poor absorption ability. Recent xanthine biosensors were based on electrochemical hybrid electrodes using Cu(II) hypoxanthine absorptive complex at a hanging mercury drop electrode (HMDE) (Pei and Li, 2000), CuPtCl₆/GC (Hu et al., 2000), sodium montmorillonite-methyl violate carbon paste (Mao et al., 2001), carbon fiber microelectrodes (CFMEs) using nafion and Au–colloid polypyrrole layer (Liu et al., 2004). These biosensors were also suffered from limited electron communication, complexity of immobilization and stability of enzymes. Ever since the conducting polymers were introduced by Diaz et al. 1979 by depositing thin films of polypyrrole (PPy) on an electrode using electro-oxidation, conducting polymers have
attracted the attention of several workers. Currently, an intensive attention is being paid to the design of nano-devices, due to the growing miniaturization of microelectronic devices. Certain conducting polymer and nanoparticles (NPs) composites have already been reported in the literature, such as PPy/Pt (Bose and Rajeshwar, 1992), PPy/Pd (Cioffi et al., 2000), PPy/TiO$_2$ (Liu et al., 2004), PPy/Ag (Liu et al., 2006), PPy/Ti, PPy/Au (Chen et al., 2007) and polyaniline/metaloxide NP composites (Nabid et al., 2008; Phang et al., 2008; Moghaddam et al., 2009).

Polypyrrole is a frequently studied conducting polymer due to its application in sensing and catalysis. Polypyrrole is considered among the most promising conductive polymers due to its stability and ease of conversion between conducting and insulating forms. Different chemical and electrochemical methods are generally used in the synthesis of polypyrrole. Despite many interesting applications, the use of polypyrrole is limited because of difficulty in processing it. Several approaches have been explored to improve the ability to process polypyrrole, including the use of emulsion, inverse emulsion, steric stabilizer, and microemulsion methods (Skotheim et al., 1998).

Nanostructure metal oxides are known to have unique ability to promote faster electron transfer kinetics between electrode and the active site of the desired enzyme (Zhou et al., 2005; Wang et al., 2006a; Singh et al., 2007; Kumar and Chen, 2008; Pandey et al., 2008). Among the metal oxide nanoparticles, ZnO-NPs have been exploited as a potential material for biosensing, because of their unusual properties i.e., high surface area for strong adsorption (high isoelectric point 9.5), good biocompatibility, chemical stability, non-toxicity and high electron communication. Nanoporous ZnO greatly enhances the surface area for strong adsorption of biomolecules (Wang et al., 2006a: Singh et al., 2007). These nonporous ZnO-NPs/ZnO nanorods/nanocombs have been employed for the fabrication of different biosensors such as uric acid (Zhang et al., 2004), microperoxidase, hydrogen peroxide (Zhu et al., 2007), glucose (Wang et al., 2006a), myoglobin (Zhao et al., 2006), phenol (Li et al., 2006), human IgG (Wang et al., 2006b) and cholesterol (Khan et al., 2008).

To the best of our knowledge, no attempts have been made towards the development of a xanthine biosensor based on zinc oxide nanoparticles-polypyrrole
composite (ZnO-NPs/PPy). We report here in the construction of a novel amperometric xanthine biosensor by immobilizing commercial XOD from buttermilk on ZnO-NPs/PPy composite film electrodeposited onto Pt electrode and its application in fish meat and serum. Hence the present study includes the following aims and objectives:

i. Preparation of zinc oxide nanoparticles (ZnO-NPs)

ii. Preparation of zinc oxide nanoparticles /polypyrrole film modified Platinum (ZnO-NPs/PPy/Pt) electrode.

iii. Immobilization of xanthine oxidase on zinc oxide nanoparticles /polypyrrole film modified Platinum electrode (XOD/ZnO-NPs/PPy/Pt).

iv. Construction and testing of xanthine biosensor.

v. Optimization of xanthine biosensor

vi. Evaluation of xanthine biosensor

vii. Interference study of xanthine biosensor

viii. Application of xanthine biosensor for determination of xanthine in fish meat and serum.

ix. Reusability and storage stability of biosensor.