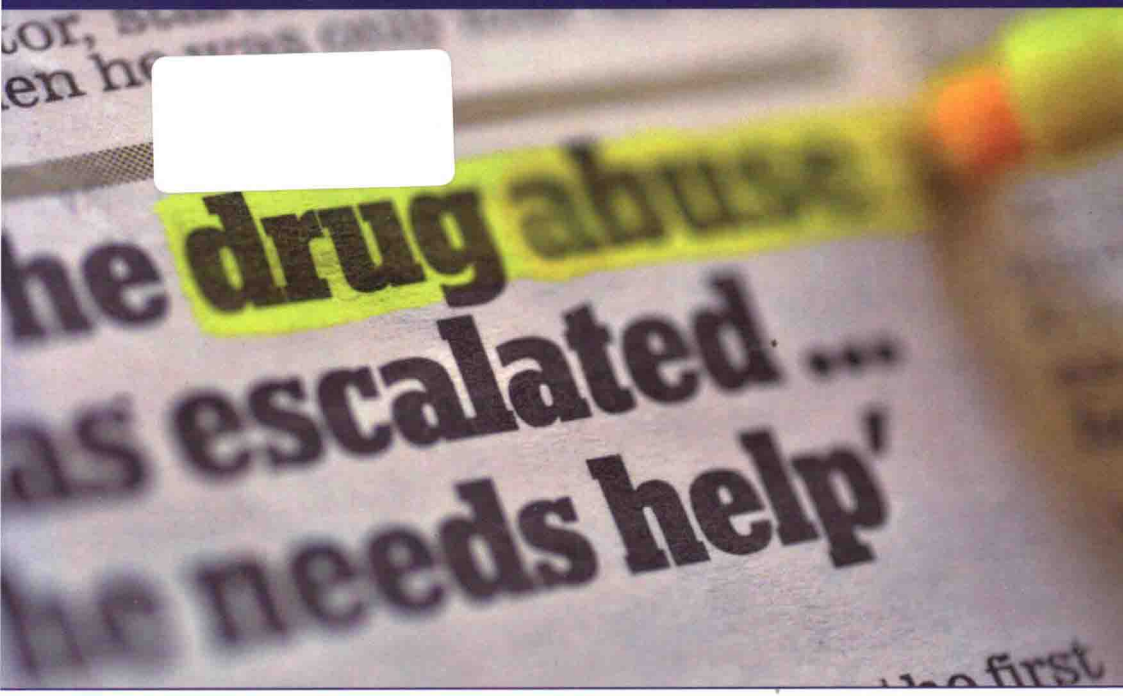

Sonu Gandhi

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(Sonu Gandhi)

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List of Abbreviations

MAM	6-monoacetyl morphine
MAM-D	Derivative of 6-monoacetyl morphine
3D	Three dimensional
AFM	Atomic Force Microscopy
AMBER	Assisted Model Building and Energy Refinement
APTES	Aminopropyltriethoxy silane
AR	Analytical Reagent
BSA	Bovine serum albumin
Bp	Base Pair
DNA	Deoxyribonucleic acid
dNTP	2'- deoxyribonucloside 5'-triphosphate
g	Gram
h	Hour
i.e.	Id est
kb	Kilobase pairs
KDa	Kilo Dalton
l	Litre
Ty	Tryptone yeast extract
M	Molar
mg	Milligram
min	Minute
ml	Millilitre
mM	Millimolar
MW	Molecular Weight

ng	Nanogram
nm	Nanometer
°C	Degree Celsius
pM	Pico molar
PCR	Polymerase chain reaction
RNase A	Ribonuclease A
rpm	Revolution per minute
RT	Room temperature
sec	Second(s)
TEMED	N'N''-tetramethylethylenediamine
Tris	Tris (hydroxymethyl aminomethane)
U	Unit
RU	Response Unit
UV	Ultra violet
V	Volt
w/v	Weight per volume
µg	Microgram
µl	Microlitre
µM	Micromolar
CD	Circular Dichroism
CFA	Complete Freund's adjuvant
CNDO	Complete Neglect of differential Overlap
sulfo-NHS	sulfo N-hydroxysuccinimide
DMF	Dimethyl formamide
DMSO	Dimethy sulfoxide
DSSP	Dictionary of secondary structure of proteins
ELISA	Enzyme Linked Immunosorbant assay
GAFF	General Amber force field
GC	Gas Chromatography
H_2O_2	Hydrogen Peroxide

HPLC	High performance liquid chromatography
HRP	Horse radish peroxidase
KHz	Kilo Hertz
IC_{50}	Inhibitory concentration giving 50% decrease of maximum signal.
IFA	Incomplete Freund's adjuvant
Ig	Immunoglobulin
IR	Infra Red
LD_{10}	Least detection limit, analyte concentration giving 10% decrease in signal
LFDA	Lateral Flow Dipstick Assay
MALDI-TOF	Matrix Assisted Laser Desorption Ionization-time of flight
MS	Mass spectrometry
NMR	Nuclear Magnetic Resonance Spectroscopy
OD	Optical Density
PAGE	Polyacrylmide gel electrophoresis
PB	Phosphate buffer
PBS	Phosphate buffered saline
PBST	PBS containing 0.05% v/v Tween-20 detergent
PDB	Protein Data Bank
PEG	Polyethylene glycol
QSAR	Quantitative structure-activity relationships
QCM	Quartz Crystal Microbalance
RIA	Radio immunoassay
SDS	Sodium dodecyl sulfate
SPR	Surface Plasmon Resonance
TBS	Tris buffered saline
TEM	Transmission electron microscopy
TLC	Thin layer chromatography

TMB	Tetra methyl abezediene
TNBS	Trinitrobenzene sulfonic acid
Tris	Tris (hydroxymethyl) amino methane
WHO	World Health Organization
OVA	Ovalbumin form Chicken egg
CNT	Carbon nanotubes
LG-FET	Liquid gated field effect transister
FIA	Fluoroimmunoassay
CIA	Chemilumenescence immunoassay
KLH	keyhole lymphet heamocyanin
EDAC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
OD	Opitical density
AuNPs	Gold nanoparticles
Ab	Antibody
Ag	Antigen
LC	Liquid chromatography
ppb	Parts per billion
ppm	Parts per million
LOD	Limit of detection
SDS	Sodium docecyl sulfate
V_h and V_l	Variable heavy and light chain
K_{aff}	Relative affinity constant
I_d	Drain current
BCA	Bicinchoninic Acid

Introduction

Narcotic Drugs

Heroin: Drug abuse

Heroin is a synthetic derivative of morphine, a naturally occurring substance extracted from unripe seeds or capsules of *Papaver somniferum* (Poppy plant). The white crystalline form of heroin is 3, 6- diacetyl derivative of morphine and is synthesized by acetylation. Heroin can exist in two forms: an insoluble base and a soluble salt. The physical appearance of illicit heroin varies widely, ranging from almost pure white heroin hydrochloride intended for injection (and often indistinguishable from pharmaceutical grade diamorphine) to crude and impure heroin, which is often in the base form and is intended for smoking or inhaling. The types of heroin encountered, listed by source of supply are described below (O'Niel et al., 1984).

South-East Asia

(a) Chinese No.3: a hard granular material (usually 1 to 5 mm in diameter), that does not yield to pressure and often contains only small amount of powder varying in color from grey to dirty brown.

(b) Chinese No. 4: a white microfine dry powder, often crystalline.

(c) Panang Pink: a granular material similar to Chinese No. 3 but dirty pink in colour. In Chinese No. 4 alkaloids are always present as the hydrochloride salts. The other two types most often occur as hydrochloride but may be the free base(s).

Drug abuse is a complex phenomenon, which has various social, cultural, biological, geographical, historical and economic aspects (O'Niel et al., 2001). Drug use, misuse or abuse is also primarily due to the nature of the drug abused, the personality of the individual and addict's immediate environment. The fast changing social milieu, among other factors, is mainly contributing to the proliferation of drug abuse, both of traditional and new psychoactive substances.

The introduction of synthetic and intravenous drug use, leading to HIV/AIDS, has added a new dimension to the problem, especially in the Northeast states of the country. Inhalation of heroin alone has given way to intravenous drug use in combination with other sedatives and painkillers. This has increased the intensity of the effect, hastened the process of addiction and complicated the process of recovery. Cannabis, heroin, and pharmaceutical drugs are the most frequently abused drugs in India. The International Narcotics Control Board (2002) report released in Vienna, pointed out that in India persons addicted to opiates are shifting their drug of choice from opium to heroin. The intravenous injections of analgesics like dextropropoxphene etc. are also reported from many states, as it is easily available at the $1/10^{th}$ cost of heroin. The codeine-based cough syrups continue to be diverted from the domestic market for abuse.

The use of heroin and morphine as a recreational drug has reached epidemic proportions, largely because of increased availability (Das et al., 1993). Recent surveys report that nearly 1 million individuals abuse heroin in India and there are as many as 500 million individuals in United States (National Institute on drug abuse, 2006). Additionally, 12.4% of cocaine related emergency room involves simultaneous heroin use, making heroin one of the drugs most commonly co-abused with cocaine, second only to ethanol. From another survey, 52% of drug abusers have simultaneously abused cocaine and heroin on one or more occasion and 5 to 12% co-administer these substances on a daily basis (Siegel et al., 1984 and 1986).

Drug Trafficking: Indian Scenario

At the national level, drug abuse is intrinsically linked with racketeering, conspiracy, corruption, illegal money transfers, terrorism and violence threatening. Several measures involving innovative changes in enforcement, legal and judicial systems have been brought into effect. The Narcotic Drugs and Psychotropic Substances Act, 1985, were enacted with stringent provisions to curb this menace. The Act envisages a minimum term of 10 years imprisonment extendable to 20 years and fine of Rs. 1 lakh extendable up to Rs. 2 lakhs for the offenders. The Act has been further amended by making provisions for the forfeiture of properties derived from illicit drugs trafficking. Comprehensive strategy involving specific

programme to bring about an overall reduction in use of drug has been evolved by the various government agencies and NGOs and is further supplemented by measures like education, counseling, treatment and rehabilitation programme. India has bilateral agreements on drug trafficking with 13 countries, including Pakistan and Burma. India also is signatory to the following treaties and conventions:

- 1961 U.N. Convention on Narcotic Drugs
- 1971 U.N. Convention on Psychotropic Substances
- 1988 U.N. Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances
- 2000 Transnational Crime Convention

India is the world's largest producer of illicit opium for the pharmaceutical trade, but an undetermined quantity of opium is diverted to illicit international drug markets. June 26 is celebrated as International Day against Drug Abuse and Illicit Trafficking every year. It is an implement undertaken by the world community to sensitize the people in general and the youth in particular, to the menace of drugs. About 190 million people all over the world consume one or the other drug. According to a UN report, One million heroin addicts are registered in India, and unofficially there are as many as five million.

Heroin Production and Use: Global Scenario

Worldwide illicit opium cultivation continued to increase in 2007, with a potential opium production of 8,400 metric tons, reaching the highest levels recorded since estimates began in mid-1980s.

Afghanistan is the world's primary opium producer (Afghanistan opium survey – 2004), accounting for about 95% of the global supply (Afghanistan, Time Magazine Asia, 2006), Southeast Asia responsible for 9% of global opium production, Latin America produced 1% of global opium, but most was refined into heroin destined for the US

market, if all potential opium was processed into pure heroin, the global production would be 1,000 metric tons of heroin in 2007.

According to UNDCP, annual illicit drug consumption is likely to involve 3 to 4% of the world's population. According to UNESCO, the international drug trade is now worth $400 billion annually, ranking second in turnover only to the arms industry. Usually a pound of heroin can cost from $US 5000-7000, depending on the quality and purity of the drug. It is illegal to manufacture, possess or sell heroin in the United States but, under the name of diamorphine, heroin is a legal prescription drug in the United States. In 2004, worldwide potential illicit opium production increased to 5,361 metric tons, from 3,549 metric tons during 2003. The potential production in Southwest Asia in 2004 accounted for 5,020 metric tons and production in Southeast Asia accounted for 341 metric tons (International Narcotics Control Board, 2004). The drug trade has become a truly global industry and an organized business being carried out with the support of huge capital, manpower, means of transportation, expertise, influence and power.

The seizure of illicit drugs, knowledge of their probable origin and unfolding the distribution network are of utmost concern for the law enforcing authorities. At present, opium poppies are mostly grown in Afghanistan, and in Southeast Asia, especially in the region known as the Golden Triangle straddling Myanmar, Thailand, Colombia, Vietnam, Sinaloa region of Mexico, Laos and Yunnan province in the People's Republic of China. The majority of the heroin consumed in the United States comes from Mexico and Colombia.

The spread and entrenchment of drug abuse needs to be prevented, otherwise the cost to the people, environment and economy will be colossal. Therefore, there is a need for the government enforcement agencies, the non-governmental philanthropic agencies, and others to collaborate and supplement each other's efforts for solution to the problem of drug addiction through education and legal actions.

Methods of drug detection

The currently used techniques viz. Thin Layer Chromatography (TLC), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and Infrared

Spectroscopy (IR), Mass Spectroscopy (MS), Nuclear Magnetic Resonance (NMR) based methods and immunoassays like RIA (radioimmunoassay) and ELISA (enzyme linked immunosorbant assay) for the detection of opiate drugs are time consuming, expensive and not amenable to on-site application. Due to the various shortcomings of current methods available for drug detection, there is a need to develop analytical tools that are portable, reliable, selective and highly sensitive towards particular analyses of opiate drugs in biological sample.

Biosensors

A biosensor can be defined as a quantitative or semi quantitative analytical instrument containing a sensing element of biological origin, which is either integrated within or is in intimate contact with a physico-chemical transducer (Turner et al., 1987, 1989, 1992). The biological components used in the biosensor construction can in general be divided into two categories: those when the primary sensing event results from catalysis (e.g. enzymes, micro-organisms, cells or tissues) and those which depend on an essentially irreversible binding of the target molecule. e.g. antibodies receptors, nucleic acids (Dennison and Turner, 1995).

In this study, antibodies were chosen as the biological recognition element for the development of biosensor because the vertebrate immune system is practically capable of producing antibodies specific for any target analyte, provided it is able to initiate an immune response. Consisting of a biological detection element in close association with a physical transducer, a biosensor offers a fast method of monitoring biomolecular interactions. The specific interaction of biomolecules with target analyte generates a physical response and the transducer detects this physical change and produces a corresponding electronic signal.

Therefore, an ideal biosensor should meet the following criterias

A) Selectivity
B) Sensitivity
C) Linearity and reproducibility of signal response

D) Quick response time and recovery time

E) Stability and operating life

Various types of biosensors have been developed for the detection of opiate drugs viz:

1) Enzyme based amperometric heroin biosensor (Holt et al., 1995, Holt et al., 1996).

2) Enzyme sensors for morphine and codeine based on morphine dehydrogenase and laccase. (Bauer et al., 1999).

3) A recombinant single chain variable fragment (scFv) antibody to morphine-3 – glucuronide (M_3G) produced, using genetic material obtained from the spleen cells of mice immunized with M_3G-BSA conjugate (Dillon et al., 2003).

4) Phage display of recombinant antibodies in making antibodies against 6-acetylmorphine (Moghaddam et al., 2003).

5) A flow injection electro generated chemiluminescence (ECL) based method have been developed for the detection of heroin using tris (2, 29-bipyridyl)ruthenium(II) $(Ru(bpy)_3^{2+})$ (Zhuang et al., 2005).

6) Lipoate derivatives for the formation of imprinted self-assembled molecular thin films for the recognition of morphine (Tappura et al., 2007).

7) Surface Plasmon Resonance (SPR) based inhibition immunoassays to characterize the ability of the free drug (morphine-3-glucuronide) (Brennan et al., 2003; Dillon et al., 2003).

To the best of our knowledge no work on the antibody based biosensors for the detection of opiate drugs has been carried out in India. Analytical methods for the detection of heroin and its metabolites vary from relatively facile chemical colour tests and TLC to more sophisticated and costly instrumental techniques like GC-MS and HPLC. Whereas GC and HPLC are routinely employed for heroin and morphine analysis, native fluorescence is one of the most sensitive method for the detection of morphine and some related alkaloids, prior to chromatographic separation of the sample constituents. Also, antibody based techniques and kits are available, but no acceptable system as yet for heroin and morphine detection has been developed. Therefore, there is a need to develop a biosensor which could detect these drugs on site and is cost effective also. The present