Gandhi *et al.*, ***BioImpacts,*** 2015, 5(4), 207-213
doi: 10.15171/bi.2015.30
http://bi.tbzmed.ac.ir/

# Recent advances in immunosensor for narcotic drug detection

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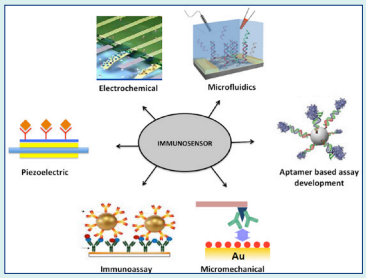
**Article Info**

***Article Type:***
Review

***Article History:***
Received: 03 Dec. 2015
Revised: 19 Dec. 2015
Accepted: 26 Dec. 2015
ePublished: 28 Dec. 2015

***Keywords:***
Narcotic drugs
Immunosensor
Transducer
Aptasensor
Immunoanalytical techniques

**Abstract**




***Introduction:*** Immunosensor for illicit drugs have gained immense interest and have found several applications for drug abuse monitoring. This technology has offered a low cost detection of narcotics; thereby, providing a confirmatory platform to compliment the existing analytical methods.

***Methods:*** In this minireview, we define the basic concept of transducer for immunosensor development that utilizes antibodies and low molecular mass hapten (opiate) molecules.

***Results:*** This article emphasizes on recent advances in immunoanalytical techniques for monitoring of opiate drugs. Our results demonstrate that high quality antibodies can be used for immunosensor development against target analyte with greater sensitivity, specificity and precision than other available analytical methods.

***Conclusion:*** In this review we highlight the fundamentals of different transducer technologies and its applications for immunosensor development currently being developed in our laboratory using rapid screening via immunochromatographic kit, label free optical detection via enzyme, fluorescence, gold nanoparticles and carbon nanotubes based immunosensing for sensitive and specific monitoring of opiates.

## Introduction

Heroin is a diacetyl ester of morphine, isolated from seeds of poppy plant *(Papaver somniferum)*. The widespread uses of these drugs cause major health related illnesses all over the world. The development of specific, reliable and simple methods to detect illicit drugs in biological samples are utmost requirement.[1,2] Analytical methods for the monitoring of opiates viz. such as heroin and its analogues employs from relatively simple chemical color tests and thin-layer chromatography (TLC) to complex instrumentation techniques e.g. gas chromatography in association with mass spectrometry (GC-MS). The gas chromatography (GC) and high performance liquid chromatography (HPLC) techniques have proliferated with time for continuous measurement of heroin and morphine.[3-8] However, these established techniques have various drawbacks as available methods are expensive, time consuming, need many cleanup steps, and also not amenable to on site applications. Therefore rapid screening methods are required for monitoring of opiate drugs. Immunoanalytical techniques offer great advantage with simple, robust and sensitive detection of analytes by targeting specific antibodies. These analytical techniques include a wide variety of immunoassay based approaches such as optical, piezoelectric, micromechanical, electrochemical, aptameric etc. Immunoassay based on the specific interaction of antigen and antibody is widely used for the immunosensor development of opiate drugs. Here we summarize the basic concept of immunosensor development by discussing its bimolecular traits and further explaining different types of transducer and aptamer based approaches that utilize antigen and antibodies for immunosensing of the opiate drugs.

## Bimolecular traits for the development of immunosensor

Since molecular weight of opiate drugs are less than 1000 Daltons, therefore immunocomplex (antigen- antibody) formation on the surface of transducer with hapten mol-

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ecules in immunoassay development does not generously alter the physical properties (mass/optical/electro-chemical) of transducer device. These molecules are therefore coupled with carriers such as protein, enzyme or fluorescence and used as tracer for immunoassay development in screening and selection of antibodies.[9-11] For immunosensor based monitoring of opiate drugs, it is essential to develop antibodies with broad specificity and high sensitivity towards target analyte.

To increase the specificity and sensitivity of the immunoassay for narcotics, it is imperative to functionalize and conjugate the hapten with carrier protein to generate specific antibodies against hapten. Therefore, it is pertinent to selectively choose the specific group of hapten that will be utilized further for conjugation with protein molecule by covalent bonding to mimic the structure of carrier protein. In our case, we used monoacetyl morphine (MAM) hydroxyl group for the functionalization with acidic reaction that results in the modification of hydroxyl group to carboxylic acid derivative of MAM. The carbodiimide activation was done to conjugate derivatized MAM hapten to carrier protein BSA (bovine serum albumin). The interactions that occur during this process mainly involve the electrostatic or hydrophobic, followed by the formation of amide bond between amino groups of protein and carboxylic groups of hapten. We have explained the above-mentioned mechanism by schematic illustration in Fig. 1. Furthermore, it has already been reported that optimum number of hapten molecules per protein resulted in the generation of antibodies with high specificity and sensitivity.[9] In conclusion, the conjugation of hapten with carrier protein is an important parameter for specific and sensitive immunoassay development.

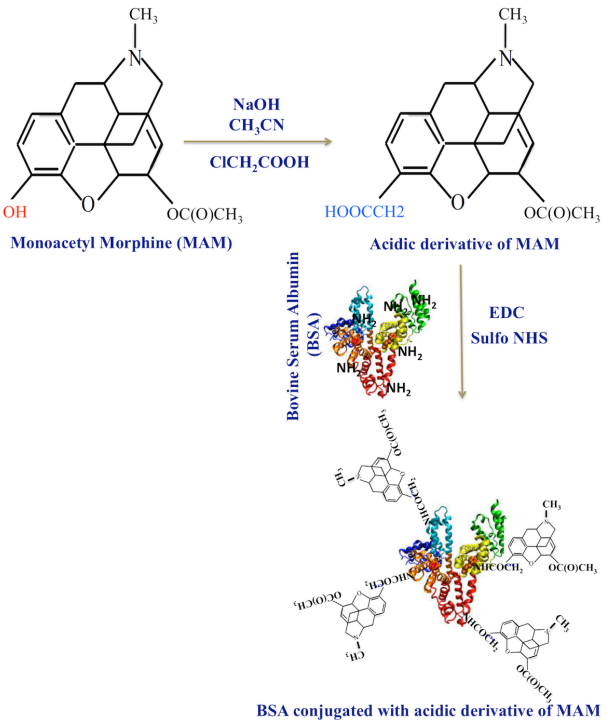


**Fig. 1.** Chemical synthesis of acidic derivative of monoacetyl morphine (MAM) followed by conjugation with carrier protein bovine serum albumin (BSA).

The competitive immunoassay format measures the competition among labeled and unlabeled analyte to bind with the available binding sites of antibodies to be monitored on the surface of transducer by binding to the immobilized antigen. This can also be achieved by Ag immobilization on the transducer surface.

Various assays have been developed for different transducer based immunosensor using polyclonal, monoclonal and scFv antibodies against different hapten molecules e.g. heroin, morphine, monoacetyl morphine (MAM), morphine-3-glucuronide ($M_3G$). Fig. 2 shows the immunosensor development by various methods such as polyclonal, monoclonal and single chain fragment variable antibodies (scFv). Polyclonal antibodies have greater advantage where we can have large pool of antibodies in its native conformation, therefore, are more stable due to presence of heavy chain in the constant region. Besides polyclonal antibodies, monoclonal Abs (mAbs) of desired affinities can also be screened. Hybridoma technique also serves an attractive alternate, where mAbs can be synthesized in unlimited quantity with consistent characteristics. In line up with the above statement, phage display technology is now widely used in making recombinant antibodies for clinical, therapeutic and diagnostic applications. The major advantage of phage display in comparison with the available hybridoma technology based methods is the specificity of the scFv/Fab fragments towards a specific antigen that can be generated in a short span of time, isolation and production is cheaper than monoclonal antibodies, animal immunization, sacrification and bleeding can be avoided. Therefore, the proposed approaches can be used as powerful tool to develop immunosensor to explore the possibilities in diagnostic applications.

## Transducer based immunosensors for opiate analysis

Immunosensors are analytical tools in which the electrical signal can be processed, recorded and displayed due to the formation of antigen-antibody (Ag-Ab) complex (Fig. 3). Transducers based approaches depend on signal generation (viz. electrochemical or optical) or property changes (such as change in mass) due to the formation of Ag-Ab complex. There are several types of transducers for opiate detection that will be discussed with some detail in this review, the central ones being optical, piezoelectric, micromechanical, and electrochemical (as summarized in Table 1).

### *Optical immunosensor*

Optical immunosensor measures the change in optical properties at the surface of transducer due to Ag-Ab complex formation. The change in the optical characteristics of the transducer depends on measurement mode i.e. direct or indirect. The signal generated in the direct optical measurement is due to the formation of immunocomplex. The indirect signal generated due to labels (flurophore or chromophore) and produce better signal to noise percep-

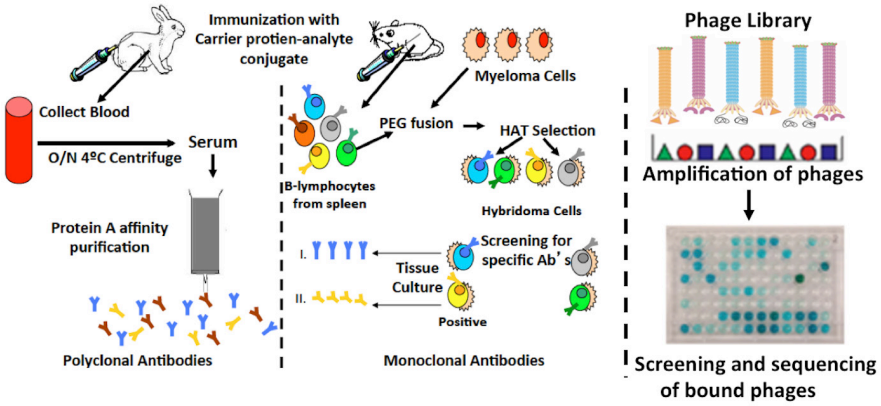


**Fig. 2.** Schematic displaying various methods of antibody development (a) Polyclonal, (b) Monoclonal, (c) phage antibody library and stepwise selection of binders for selective antigen.

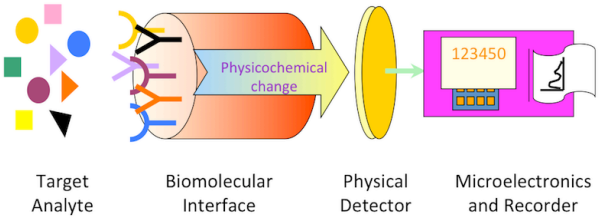


**Fig. 3.** Schematic representation of the biosensor development. The biomolecular interface interacts with the target analyte that subsequently leads to physicochemical changes on the biomolecular interface. These changes are further detected by the detector and recorded by the recorder.

tion. An indirect fluorescence based competitive immunoassay was developed in our laboratory for the monitoring of monoacetyl morphine (MAM) with sensitivity in pg/ml.[9] In continuation of the enzyme and fluorescence based methods colloidal gold based rapid immunochromatographic dipstick kit was also developed with sensitivity upto ng/ml. The developed immunoassay was rapid, amenable to on site with no prerequisite of sophisticated instrumentation.[10] Similar indirect fluorocompetitive assays were also developed for other opiate drugs such as papavarine (PAP) and morphine[12,13] and its metabolites by using an immunoaffinity column based approaches.[14]

The SPR (Surface Plasmon Resonance) based immunoassays are extremely specific and sensitive for the monitoring of wide variety of hapten molecules (usually less then 1000 Da).[15] A novel inhibition assay was developed with sensitivity in ng/ml, using SPR immunosensor for $M_3G$, which is a major metabolite of heroin and morphine.[16,17] Another cost effective method was developed for $M_3G$ and morphine where SPR chip was regenerated upto 15 cycles,[18-20] lipoate as novel detection tool for morphine,[21] label free detection of heroin, cocaine, ecstasy and amphetamine with the detection limits in pg/ml.[22]

### *Piezoelectric immunosensors*

The main principle underlies is the change in mass at the surface of quartz crystal leading to change in oscillating frequency due to the formation of immunocomplex. A piezoelectric crystal is intervened between two electrodes that measure small changes in mass on the crystal surface due to the formation of immunocomplex. The change in oscillating frequency (ΔF) at the crystal surface is due to change in the mass (Δm) that can be explained by Sauerbrey equation:[23]

$\Delta F = -kF^2\Delta m/A^{-1} = -2{:}26 \times 10^{-6} F^2 \Delta m/A^{-}1$

where, ΔF is the frequency change (Hz),

k is the proportional constant that depends upon Density and Shear modulus of piezoelectricquartz crystal (density of quartz = 2.648 g/cm$^3$ and Shear modulus of quartz = 2.947 X 10$^{11}$g.cm$^{-1}$.s$^{-2}$), F is resonant frequency (MHz) of quartz crystal, Δm is change in mass (g), and A is area of piezoelectriccrystal (cm$^2$).

A monoclonal antibody for benzoylecgonine (BE), that is main metabolite of cocaine, was coated on quartz fibers for quantification of cocaine by piezoelectric fluoroimmunosensor.[24]

### *Micromechanical immunosensors*

The formation of immunocomplex on cantilever due to distinct chemical and biomolecular interaction between ligand and receptor leads to deflection of cantilever beam in the range of nanoscale or even lower. This mechanical bending results in change in the shear stress properties due to the binding of Ag-Ab that offers quantitative measurement.

The real deflection of cantilever surface (Δz) can be monitored by optical detection in liquid state with constant flow of antigen. The deflection beam signal caused change in surface stress (Δσ), according to Stoney's equation:[25]

$\Delta\sigma = \frac{1}{4}(t/L)^2 E/(1-\nu)\Delta z$,

where L =length of the cantilever, t =cantilever thickness, and E = Young's modulus and ν = Poisson ratio

The selectivity and sensitivity of the assay is governed by molecular probe (receptor) on the cantilever surface and degree of cantilever deflection after immunocomplex formation.

Protein-protein interaction, DNA hybridization and other chemical binding processes can be detected by label-free cantilever based sensor, due to thermodynamic source

and amenability to traditional fabrication procedures. In recent study, microcantilever based sensor was used for detection of cocaine where cocaine specific aptamer was used as receptor with lowest detectable threshold in μM range.[26]

### *Electrochemical immunosensors*

Electrochemical sensors are established for the measurement of amperometric, potentiometric, conductimeteric and changes in the capacitance associated with the Ag-Ab interactions that lead to change the in current, potential, conductance and capacitance respectively. The main principle involved is the change in the current on electrode surface due to oxidation and reduction of adsorbed analyte. The first electrochemical detection method was developed for two different set of enzymes heroin esterase and morphine dehydrogenase.[27-30] Further, a series of experiments in continuation were done for heroin and morphine by immobilization on the electrode surface using the same enzyme heroin esterase and morphine dehydrogenase.[31,32] A label free electrochemiluminescence based immunosensor for morphine was developed with sensing threshold in ng/ml[33] while recent method was based on voltametric principle for morphine sensing by using DNA as a probe on Au electrode.[34]

### *Carbon nanotubes (CNTs) based immunosensors*

CNTs are used for its attractive electrical properties and serve as perfect model for electrodes and transducer-based biosensors due to several attractive properties such as large surface area due to large length-to-diameter ratio to facilitate good thermal conductivity by fast electron-transfer kinetics for electroactive species, enhanced solubility by chemical functionalization.[35-38] A CNT network or single CNT deposited across two metal contact pads act as the semiconducting channel of the transistor that function as the sensing interface (Fig. 4).

Numerous works have been carried out on a number of bioactive and biological species such as DNA, peptides, enzymes, proteins, antibodies and their interaction with CNTs were refined for better attachment.[39-42] Our laboratory developed CNT based immunosensor for the detection of MAM in real time with potential LOD (limit of detection) beyond fg/ml.[43]

A highly sensitive CNT based immunosensor was developed to monitor morphine and noscapine, using carbon nanotubes immobilized on preheated glassy carbon electrode that was based upon the efficient electrocatalytic oxidation.[44-46]

### *Aptasensors*

Aptamers are oligonucleotide molecules that hold high specificity and affinity towards target analyte (Fig. 5). These aptameric structures offer an exceptional advantages viz. chemical stability, imitation expediency etc. and have become progressively more powerful sensing tool for diagnostic applications. Aptameric sensors were developed for cocaine using novel electrogenerated chemiluminescence (ECL) based assay with detection limit in nM range,[47] fluoroaptameric sensor with target induced strand displacement,[48] DNA based aptamer with detection in mM,[49] single quantum dot (QD) based aptameric sensor.[50] Several bioassay strategies were established using gold nanoparticles for determination of cocaine, potassium, adenosine with engineered DNA aptamers. In presence of specific target, the aptamer was in intact tertiary conformation that was synthesized as two random coils of single stranded DNA (ssDNA). Gold Nanoparticles (AuNPs) were used to differentiate between these two forms of DNA due to change in the characteristics of SPR. This method was also used for cocaine detection in low

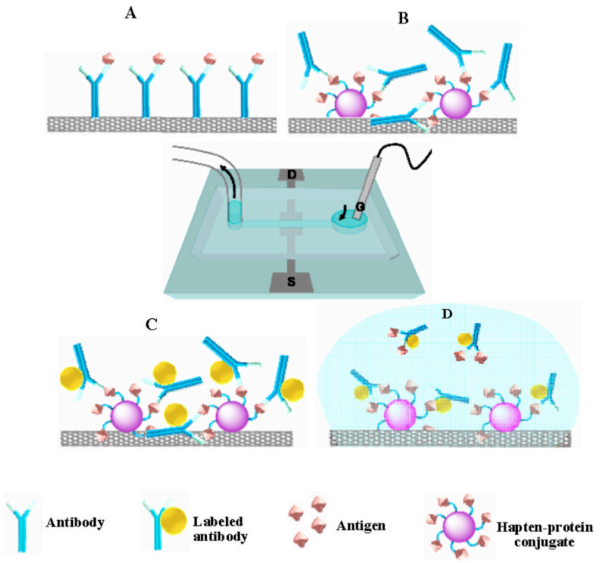


**Fig. 4.** CNT based Assay- Different schemes for the detection of opiate drugs using a carbon nanotubes (CNT) liquid gated field effect transistors; [A] Direct detection of antibodies; [B] Indirect detection of antibodies by using hapten-protein conjugate; [C] Indirect immunoassay by using labeled antibodies; [D] Indirect competitive immunoassay.

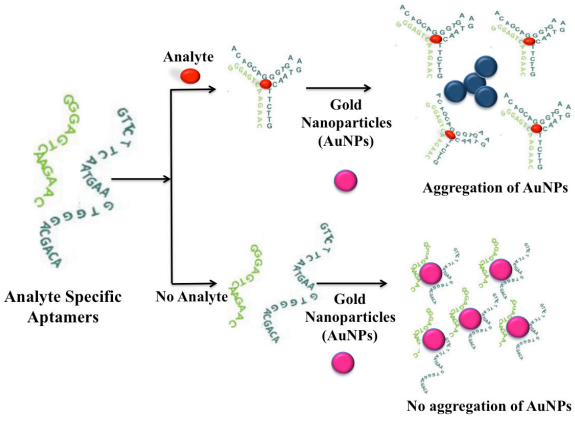


**Fig. 5.** Aptamer Based Assay- The sequence of the DNA aptamer and proposed folding pathway. The binding of target with probe triggers the formation of aptamer-target complex that results in the aggregation of gold nanoparticles (AuNPs).

**Table 1.** Examples of various types of immunosensors for opiate drugs with its detection limit

| Name of immunosensor | Drug Name | Limit of detection | Reference |
|---|---|---|---|
| Piezoelectric | Cocaine | 34 ng/l | 52 |
| | Cocaine & Ecstasy | 100 & 200 ng | 53 |
| Micromechanical | Cocaine | 1 ng/mL | 55 |
| | MDMA | 5 µg/mL | 56 |
| Electrochemical | Cocaine | 0.005 µg/mL | 63 |
| | Morphine | 0.2-1.2 mM | 58 |
| Carbon Nanotube | MAM | 15 pg/mL | 43 |
| | Morphine | 0.01 mM; 0.01 µM | 54,59 |
| Optical | Heroin & its metabolites | 1-1.4 ng/mL | 9 |
| | Opiate drugs | 2.5 ng/mL | 10 |
| | M-3-G | 3-97 ng/mL | 18 |
| | MDMA & Ecstasy | 20 ng/mL & 0.2 ng/mL | 61 |
| | Morphine | 2000 ng/mL | 60 |
| | Morphine | 0.1 nM | 64 |
| Aptamer | Cocaine | 2 nM | 48 |
| | Codeine | 3 pM | 62 |

micromolar range.[51]

### *Immunosensor for heroin and its metabolites*

The routine monitoring of opiate drugs significantly improves the efficiency of currently available simple and rapid drug analysis procedures. These methods are based on biosensors that could significantly be improved by developing sensitive and specific detection method. We have demonstrated our laboratory research about anti-MAM antibodies generated by chemical modification of hapten (MAM) and subsequent conjugation with carrier protein (BSA) for immunorecognition. Opiate drugs are hapten molecules that were synthesized, functionalized and conjugated with carrier protein to generate class-specific or compound specific Abs against target molecule to implement particular immunoassay. The carboxylic acid groups were generated by chemical method on the surface of opiate molecule (MAM) for the attachment of carrier protein (BSA) by carbodiimide chemistry to mimic the structure of anti-MAM antibodies in animal model. We further modulated the number of hapten molecules per carrier protein to selectively raise Abs with high specificity against the target analyte. We have developed several enzymes, fluorescence, CNTs and nanoparticles based immunoassays for efficient monitoring of narcotic drugs with high specificity and sensitivity.[9,10,43] The developed antibodies were group selective that provides rapid and cost effective assays for multiple analytes[9,10] with increased sensitivity upto to fg/ml.[43]

## Concluding remarks

The basic concept and promising feature of immunosensor is based on the production of specific and highly sensitive Abs against low molecular mass opiates particularly for a large pool of narcotic drugs that would help to monitor the presence of drugs in biological fluid. Immunosensors have been widely used for the monitoring of narcotic drugs in real biological specimen that provide an alternate tool for cost effective and on site monitoring of narcotic drug. In this review we discussed about various transducers based approaches that provide the better understanding of measurement of immunoassays as an analytical device for on-site monitoring of opiate drugs. We have successfully developed immunochromatographic kit as an alternate optical method that could be used for the analysis of opiates and its metabolites in biological fluids. Further studies should be performed to develop immunosensor for multianalyte monitoring and optimizing the sensitivity of the developed assay by changing various parameters such as buffer conditions, optimum labeling, pH to increase the sensitivity for the rapid and on-site assays.

## Acknowledgments

Authors would like to thank Dr. Prem Tripathi (Seattle Children Hospital, Seattle, USA) for valuable comments. We are also very grateful to Central Forensic Science Lab-

### Review Highlights

**What is current knowledge?**

√ Antibodies have been used for sensing of analytical and diagnostic tool and named as "Immunosensors".

√ Antibodies are used for compound-specific and also for class-specific detection of opiates that utilizes its epitope region that has specific affinity for wide range of antigen (opiates).

**What is new here?**

√ Synthesis of suitable hapten (opiate) molecule for conjugation with carrier protein, to mimic the structure of opiate, can be used to generate desired Abs with high specificity and sensitivity for the development of robust immunoassay.

√ Multianalyte monitoring of opiate and its metabolites can be used for rapid screening for really useful immunobiosensor system.

oratory (CFSL) for providing us narcotic drugs for experimentation.

## Ethical issues

No ethical issues to be declared.

## Competing interests

There is none to be disclosed.

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