Amit K. Yadav,1 Damini Verma,1, 2 G. B. V. S. Lakshmi,1 Sergei Eremin,3 Pratima R. Solanki1\*

**Amine functionalized transition metal dichalcogenides based nanoparticles towards label-free immunosensing platform for antibiotics detection in food samples**

Email ID: partima@mail.jnu.ac.in

**Abstract**

The World Health Organization (WHO) has been reported that antimicrobial-resistant (AMR) bacteria have become the basic risk to human health caused due by declining potency and excessive use of antibiotics [1, 2]. During clinical/veterinary practice, antibiotics have selective pressure on bacteria that speeds up the bacterial development having AMR properties owing to pharmaceutical accumulation in the water systems and food chains. The annual mortality rate related to infections from antibiotic-resistant bacteria is as much as nearly 23,000 deaths in the U.S., according to a report of the Centers for Disease Control and Prevention [3]. Recent studies have revealed that antibiotics with enormous concentrations are found in India's drinking water, surpassing the maximum regulatory limits (MRLs). This has led to a severe effect on susceptible residents existing close to industrial services, as nearly 58,000 new-borns die in these areas of India from multi-drug resistant infections [4-6]. The tetracyclines, sulfonamides, macrolides, fluoroquinolones, and β-lactams classes of antibiotic compounds are often present in aquatic environments [7]. Ampicillin (AMP) is one of the most used -lactam antibiotics, a water-soluble chemical substance. It is extensively used in agriculture, livestock, poultry, aquaculture, veterinary, and human medicine to prevent and treat contagious diseases [8] due to the remarkable destroying ability for gram-negative and positive bacteria [9]. The overdose of antibiotics or antibiotic residues can cause severe environmental and food safety concerns that triggered various human and animal health complications in past decades involving endocarditis, membranitis, intestinal infection, and irritability [8, 10]. Therefore, detecting AMP residues in foodstuffs is essential for protecting human health as it can lead to adverse reactions, comprising anaphylactic disorders. Hence, there is an urgent need to develop reliable, sensitive, portable, inexpensive, and fast detection methods for detecting AMP, specifically.

Numerous commercial diagnostic tests are currently available for antibiotics in food samples and environments, either unable to detect antibiotic compounds selectively or need a lengthy response time (several hours) and offer semi-quantitative information only [11]. Over the decades, several attempts have been tried for developing various effective techniques for the detection of AMP residues in waters and agricultural products, which includes Raman spectroscopy [12], high-performance liquid chromatography (HPLC) [13], liquid chromatography-mass spectrometry/ mass spectrometry (HPLC-MS/MS) [14], surface plasmon resonance (SPR) [15], spectrophotometry [16], colorimetric methods [17], electrochemiluminescence [18], enzyme-linked immunosorbent assay (ELISA) [19], nuclear magnetic resonance [20], and immunochemical detection [21]. Such methods are sensitive to AMP detection, but due to high cost, sophisticated instrumentation, complicated sample preparation, and complex instrument operation, it restricts its practical applications for the on-site assays. Therefore, simple, rapid, miniaturized, and low-cost electrochemical immunosensor hold considerable attention for detecting antibiotics [22, 23]. By combining the high selectivity of antibodies with the highly sensitive transducers, antibodies-based sensors, i.e., immunosensors, have become an effective analytical technique for antibiotic residues determination [24, 25]. Also, antibody usage enhances the efficiency of the immunochemical response due to faster assay kinetics along with an increased surface area and also minimizes matrix effect with improved washing and separation steps [26, 27].

To address the antibiotic resistance problem, here we aimed to fabricate a label-free immunosensing platform for the first time based on nanostructured molybdenum disulfide nanoparticles (nMoS2 NPs) deposited on Indium tin oxide for the electrochemical detection of Ampicillin (AMP). The stable and high surface area nMoS2 NPs were made by a low-temperature one-step hydrothermal route, bestowing the carrying capacity of monoclonal antibodies (anti-AMP) through an amide linkage. Bovine Serum Albumin (BSA) was utilized to block the non-specific sites on the immunoelectrode surface. The spectroscopic, morphological, and structural characterization of the proposed electrodes were performed using various analytical techniques like X-ray diffraction (XRD), Raman spectroscopy, Fourier transform-infrared spectroscopy (FT-IR), Field emission-scanning electron microscopy (FE-SEM), Transmission electron microscopy (TEM), Atomic force microscopy (AFM) and electrochemical techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The DPV method was utilized to evaluate anti-AMP and AMP interaction on the electrode surface. The change in the MoS2 interface current was caused by the specific binding of AMP to the antibodies immobilized onto the nMoS2 NPs/ITO electrode. Other antibiotics such as norfloxacin, ofloxacin, and ciprofloxacin were used in this experiment to test the specificity of the immunosensors. The developed immunosensor exhibits high sensitivity [41.81 A (log g mL-1)-1], a broad detection range (0.0325-64 g mL-1) having a significant detection limit (0.028 g mL-1) towards detection of AMP having excellent selectivity, acceptable stability, and reproducibility. Furthermore, the applicability of the proposed immunosensor was tested in spiked milk, water, and orange juice, and the results confirmed the consistency of the immunosensor. This work shows the future potential of nMoS2 NPs in developing immunosensors for sensitive detection of antibiotics and other analytes with low cost that might prove beneficial for the on-site detection of chemical or biological molecules.



**Fig.1.** **[A]**. Schematic representation of the synthesis of nMoS2 NPs and development of BSA/anti-AMP/APTES/nMoS2/ITO immunoelectrode for AMP detection. **[B]**. (a) Electrochemical response analysis of BSA/anti-Amp/APTES/nMoS2/ITO immunosensor versus concentration of AMP (0.0325-256 gmL-1); (b)enlarge view of response study; (c)Calibration curve of BSA/anti-Amp/APTES/nMoS2/ITO immunoelectrode between peak current and concentration of AMP; and (d) Hens-Wolf plot for BSA/anti-AMP/APTES/nMoS2/ITO immunoelectrode.

**Table 1.** Recovery of AMP and their RSD from milk, orange juice, and tap water samples using BSA/anti-AMP/APTES/nMoS2/ITO immunoelectrode.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample | Concentrations added (g/mL) | DPV current for Amp (A) | DPV current for spike-sample (A) | RSD (%) | Recovery (%) |
|  Milk | 0.0625 | 105.65 | 104.61 | 0.70 | 99.01 |
| 0.5 | 95.64 | 93.170 | 1.85 | 97.41 |
| 2 | 89.51 | 87.71 | 1.44 | 97.98 |
| 8 | 83.68 | 82.89 | 0.67 | 99.05 |
| 16 | 80.02 | 82.21 | 1.91 | 102.73 |
| 64 | 75.16 | 76.29 | 1.06 | 101.5 |
| Orange Juice | 0.0625 | 105.65 | 102.81 | 1.93 | 97.32 |
| 0.5 | 95.64 | 92.163 | 2.62 | 96.36 |
| 2 | 89.51 | 86.914 | 2.08 | 97.09 |
| 8 | 83.68 | 81.542 | 1.83 | 97.44 |
| 16 | 80.02 | 81.176 | 1.01 | 101.44 |
| 64 | 75.16 | 72.509 | 2.54 | 96.47 |
| Water | 0.0625 | 105.65 | 101.28 | 2.99 | 95.87 |
| 0.5 | 95.64 | 92.773 | 2.15 | 97.00 |
| 2 | 89.51 | 90.026 | 0.41 | 100.5 |
| 8 | 83.68 | 80.749 | 2.52 | 96.49 |
| 16 | 80.02 | 78.430 | 1.42 | 98.01 |
| 64 | 75.16 | 73.364 | 1.71 | 97.61 |

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| References |  |  |  |

[1] C.A. Michael, D. Dominey-Howes, M. Labbate, The antimicrobial resistance crisis: causes, consequences, and management, Frontiers in public health 2 (2014) 145.

[2] W.H. Organization, Antimicrobial resistance and primary health care, World Health Organization, 2018.

[3] J. Syska, Frieden wave-function representations via an Einstein-Podolsky-Rosen-Bohm experiment, Physical Review E 88(3) (2013) 032130.

[4] W. Hall, Superbugs: An arms race against bacteria, Harvard University Press2018.

[5] P.K. Mutiyar, A.K. Mittal, Risk assessment of antibiotic residues in different water matrices in India: key issues and challenges, Environmental Science and Pollution Research 21(12) (2014) 7723-7736.

[6] J. Fick, H. Söderström, R.H. Lindberg, C. Phan, M. Tysklind, D.J. Larsson, Contamination of surface, ground, and drinking water from pharmaceutical production, Environmental Toxicology and Chemistry 28(12) (2009) 2522-2527.

[7] N. Kemper, Veterinary antibiotics in the aquatic and terrestrial environment, Ecological indicators 8(1) (2008) 1-13.

[8] T. Wang, H. Yin, Y. Zhang, L. Wang, Y. Du, Y. Zhuge, S. Ai, Electrochemical aptasensor for ampicillin detection based on the protective effect of aptamer-antibiotic conjugate towards DpnII and Exo III digestion, Talanta 197 (2019) 42-48.

[9] B.K. Körbahti, S. Taşyürek, Electrochemical oxidation of ampicillin antibiotic at boron-doped diamond electrodes and process optimization using response surface methodology, Environmental Science and Pollution Research 22(5) (2015) 3265-3278.

[10] R. Yuan, Z. Yan, A. Shaga, H. He, Design and fabrication of an electrochemical sensing platform based on a porous organic polymer for ultrasensitive ampicillin detection, Sensors and Actuators B: Chemical 327 (2021) 128949.

[11] O. Jamieson, T.C. Soares, B.A.d. Faria, A. Hudson, F. Mecozzi, S.J. Rowley-Neale, C.E. Banks, J. Gruber, K. Novakovic, M. Peeters, Screen printed electrode based detection systems for the antibiotic amoxicillin in aqueous samples utilising molecularly imprinted polymers as synthetic receptors, Chemosensors 8(1) (2020) 5.

[12] S.J. Clarke, R.E. Littleford, W.E. Smith, R. Goodacre, Rapid monitoring of antibiotics using Raman and surface enhanced Raman spectroscopy, Analyst 130(7) (2005) 1019-1026.

[13] M.M. Abdelrahman, I.A. Naguib, M.A. Elsayed, H.A. Zaazaa, Chromatographic methods for quantitative determination of ampicillin, dicloxacillin and their impurity 6-aminopenicillanic acid, Journal of chromatographic science 56(3) (2018) 209-215.

[14] S. Bogialli, V. Capitolino, R. Curini, A. Di Corcia, M. Nazzari, M. Sergi, Simple and rapid liquid chromatography− tandem mass spectrometry confirmatory assay for determining amoxicillin and ampicillin in bovine tissues and milk, Journal of agricultural and food chemistry 52(11) (2004) 3286-3291.

[15] E. Gustavsson, J. Degelaen, P. Bjurling, Å. Sternesjö, Determination of β-lactams in milk using a surface plasmon resonance-based biosensor, Journal of agricultural and food chemistry 52(10) (2004) 2791-2796.

[16] Y.-B. Miao, N. Gan, H.X. Ren, T. Li, Y. Cao, F. Hu, Y. Chen, Switch-on fluorescence scheme for antibiotics based on a magnetic composite probe with aptamer and hemin/G-quadruplex coimmobilized nano-Pt–luminol as signal tracer, Talanta 147 (2016) 296-301.

[17] Q. Luan, X. Xiong, N. Gan, Y. Cao, T. Li, D. Wu, Y. Dong, F. Hu, A multiple signal amplified colorimetric aptasensor for antibiotics measurement using DNAzyme labeled Fe-MIL-88-Pt as novel peroxidase mimic tags and CSDP target-triggered cycles, Talanta 187 (2018) 27-34.

[18] M.J. Li, P. Jiao, M. Lin, W. He, G.-N. Chen, X. Chen, High electrochemiluminescence of a new water-soluble iridium (III) complex for determination of antibiotics, Analyst 136(1) (2011) 205-210.

[19] T. Ramatla, L. Ngoma, M. Adetunji, M. Mwanza, Evaluation of antibiotic residues in raw meat using different analytical methods, Antibiotics 6(4) (2017) 34.

[20] U.M. Reinscheid, Direct determination of ciprofloxacin in admixtures with metronidazol and ampicillin by NMR, Journal of pharmaceutical and biomedical analysis 40(2) (2006) 447-449.

[21] K. Wutz, R. Niessner, M. Seidel, Simultaneous determination of four different antibiotic residues in honey by chemiluminescence multianalyte chip immunoassays, Microchimica Acta 173(1-2) (2011) 1-9.

[22] A.R. Cardoso, A.P. Tavares, M.G.F. Sales, In-situ generated molecularly imprinted material for chloramphenicol electrochemical sensing in waters down to the nanomolar level, Sensors and Actuators B: Chemical 256 (2018) 420-428.

[23] Y. Zhou, M. Wang, H. Yin, S. Ai, Amperometric determination of the activity of protein kinase a using a glassy carbon electrode modified with IgG functionalized gold nanoparticles conjugated to horseradish peroxidase, Microchimica Acta 184(9) (2017) 3301-3308.

[24]. J.M. Fowler, D.K. Wong, H.B. Halsall, W.R. Heineman, Recent developments in electrochemical immunoassays and immunosensors, Electrochemical Sensors, Biosensors and their Biomedical Applications, Academic press2008, pp. 115-143.

[25] F. Ricci, G. Volpe, L. Micheli, G. Palleschi, A review on novel developments and applications of immunosensors in food analysis, Analytica chimica acta 605(2) (2007) 111-129.

[26] S. Solé, A. Merkoci, S. Alegret, New materials for electrochemical sensing III. Beads, TrAC Trends in Analytical Chemistry 20(2) (2001) 102-110.

[27] Y. Xu, E. Wang, Electrochemical biosensors based on magnetic micro/nano particles, Electrochimica Acta 84 (2012) 62-73.