**ImmunoAnalysis**, 2021, 1, 12
doi:10.34172/ia.2021.12
https://ia.tbzmed.ac.ir/

Mini Review

# Sensing Methods of Immunosuppressant Pharmaceutical Drugs: Calcineurin Inhibitors and Purine Synthesis Inhibitor Agents

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**ARTICLE INFO**

Article History:
Received: 23 December 2021
Accepted: 28 December 2021
ePublished: 29 December 2021

Keywords:
Fast detection
Cyclosporine
Tacrolimus
Azathioprine
Mycophenolate mofetil
Renal transplantation

**Abstract**

Today, immunosuppression is associated as a necessary protocol in renal post-transplantation follow-up to improve both patient and graft survival rates. Two typical classes of immunosuppressants that have been used in oral long-term medication are calcineurin inhibitors, cyclosporine A (CsA) and tacrolimus (TAC), and purine synthesis inhibitor agents, azathioprine (AZT) and mycophenolate mofetil (MMF). As a common feature for all of them, they have a narrow therapeutic index (TI) which may lead to toxicity or effectiveness of therapy in super therapeutic and subtherapeutic concentrations, respectively. So, despite the undisputed value of immunosuppressants, therapeutic drug monitoring (TDM) is a vital necessity for post-renal transplantation follow-up to maintain an appropriate balance between a predefined therapeutic dose and toxicity and the likelihood of adverse effects. Previously, most of the analytical methods that have been employed for immunosuppressant detection are based on immunoassay and chromatography methods. Both of these methods suffer from drawbacks such as expensiveness, time-consuming responding, lack of stability, and need of skilled persons to interpret obtained results which make them inappropriate platforms for in-situ applications. In this way, we need facile, fast responding analytical methods as like as optical and electrochemical sensors which can be developed to use in point-of-care (POC) applications. Here, we summarized the analytical methods for the determination of CsA, TAC, AZT, and MMF which are based on fast responding methods.

## Introduction

Nowadays, immunosuppression is associated as a necessary protocol in renal post-transplantation follow-up. Evidence-based recommendations acknowledge the use of immunosuppressants improved both patient and graft survival rate.[1] Generally, 6 months to one year from the induction phase, oral long-term medication is started as the maintenance phase of treatment.[2] Two typical classes of immunosuppressants that have been used as maintenance therapy are calcineurin inhibitors, cyclosporine A (CsA), and tacrolimus (TAC) and purine synthesis inhibitor agents, azathioprine (AZT) and mycophenolate mofetil (MMF).[1,3] The common feature of all of these drugs is a narrow therapeutic index (TI) which may lead to not only toxic adverse effects in supertherapeutic concentrations, but also decrease the efficiency of treatment in subtherapeutic levels.[4,5]

CsA, despite the major advances in the reduction of human kidney transplantation rejection, is associated with problems such as nonselective immunosuppression mechanism (which increases the risk of infection or malignancies) and toxicity (particularly renal toxicity).[6] Additionally, CsA pharmacokinetics is widely affected by inter-and intraindividual variability.[1] TAC is one of the well-documented agents in the prevention of graft-vs-host disease. However, the harmful adverse effects including renal damage, neurotoxicity, hypertension, etc. may limit its advantages. It is important to mention that even a low trough concentration of TAC (4–6 ng/mL) may cause nephrotoxicity.[7] AZT is a prodrug of 6-mercaptopurine, the purine base hypoxanthine analog, which has been used for immunosuppression therapy after organ transplantation since 1960.[8] Nevertheless, long-term administration of AZT can generate many complications for patients such as hepatotoxicity, low white cell counts, pancreatitis, and even raises the risk of many kinds of cancers and infections.[9] MMF is the prodrug of mycophenolic acid, recommended as a well-tolerated immunosuppressant

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drug for the prevention of graft rejection. By the way, fetal development defects and miscarriage rate are as high as pregnant women need regular control.[10]

All these drawbacks demonstrated that despite the undisputed value of immunosuppressants, therapeutic drug monitoring (TDM) is a vital necessity for post-renal transplantation follow-up. The main aim of TDM for immunosuppression therapy is to maintain an appropriate balance between a predefined therapeutic dose and toxicity and likelihood adverse effects of them.[11]

Previously, most of the analytical methods that have been employed for immunosuppressant detection are based on immunoassay and chromatography methods. Immunoassay systems have some drawbacks like expensiveness, time-consuming responding and lack of stability. Additionally, their sensitivity is usually affected by the cross-reactivity of the antibodies. While chromatography methods, despite the high sensitivity, and selectivity towards the analyses, need high-cost instruments and skilled persons to interpret obtained results.[12]

With the aim of continuous determination of immunosuppressants concentration in biological fluids, we need facile and fast responding analytical methods which can develop as an on-site detection platform for point-of-care (POC) applications. The most sophisticated methods for this purpose are optical[13] and electrochemical[10] sensors. In this review, we summarized the analytical methods for the determination of CsA, TAC, AZT, and MMF which are based on fast responding methods. We try to provide a forward-looking perspective for new studies to design new on-site detection platforms.

## Fast detection methods of calcineurin inhibitors and purine synthesis inhibitor agents

As a facile, fast determination of CsA, Marzejon et al suggested a spectroscopic method for detection of CsA in blood plasma and hemoglobin solutions. The developed label-free method can detect CsA up to 15 mg/L in both blood and hemoglobin samples. This method provides specific detection of the CsA method and which could be utilized in clinics. As the authors declared, the obtained analytical figures-of-merit propose a capability of being as a point-of-care CsA sensor for this method.[14]

In most immunosuppressive drugs structure such as CsA and TAC, there is no chromophore or fluorophore group to use optical methods directly. In such a study, Jahed et al used silver nanoparticles (AgNPs) as a colorimetric agent which is capped by dopamine nanoparticles to determine CsA in both drug formulation and human plasma samples. Among the properties of AgNPs, the high extinction coefficient and the color distinction in the aggregation are more considered in sensing studies. In this way, Jahed et al used the surface plasmon resonance (SPR) method to demonstrate the changes in absorption intensity in presence of CsA. As shown in Figure 1, the developed colorimetric probe in presence of CsA induced aggregation which subsequently cause the color change from bright-yellow to red. This sensor was able to quantify CsA concentration in plasma samples in a linear range of 0.17-0.85 μM with acceptable selectivity and rapid analysis time to provide a promising candidate for TDM analysis. However, the stability of the sensor was not reported in their study. At least, the developed optical probe was applied to determine CsA concentration in two patients with oral administration.[12]

One of the useful functional optical platforms that have been attracted particular attention for fast analysis in recent years is the fluorescent method. For instance, Mansouri et al developed an aptamer based sensor by specific aptamer for TAC, Apt122, and labeled with ATTO 647 N oligonucleotides as a fluorophore group. In fact, the 3D structure of aptamers, which are small single-stranded sequences of DNA or RNA, has a major recognition role. In addition, graphene oxide (GO) was added as a fluorescence quencher which can bind to biomolecules with hydrogen bonds or π- π stacking bonds. The detection mechanism of the developed sensor as illustrated in Figure 2. In the absence of TAC, GO and Apt122 were attached together which decreased the intensity of the fluorescence. This aptasensor exhibits a line relationship between fluorescence intensity and TAC concentrations from $4.2 \times 10^{-3}$ to 1 μM. limit of detection (LOD) developed aptasensor was as low as $2.5 \times 10^{-3}$ μM in serum samples.[15]

In another study, Almahri et al reported both spectrofluorometric and chemiluminescence techniques for the determination of AZT in pharmaceutical formulation. In the spectrofluorometric method, they used AZTs native fluorescence spectra which were optimized by the addition of the sodium dodecyl sulfate (SDS) as a surfactant. This method showed the linear response to AZT concentration from $5 \times 10^{-2}$ to $1 \times 10^{2}$ μM and the LOD was equal to $1.5 \times 10^{-3}$ μM. The chemiluminescence

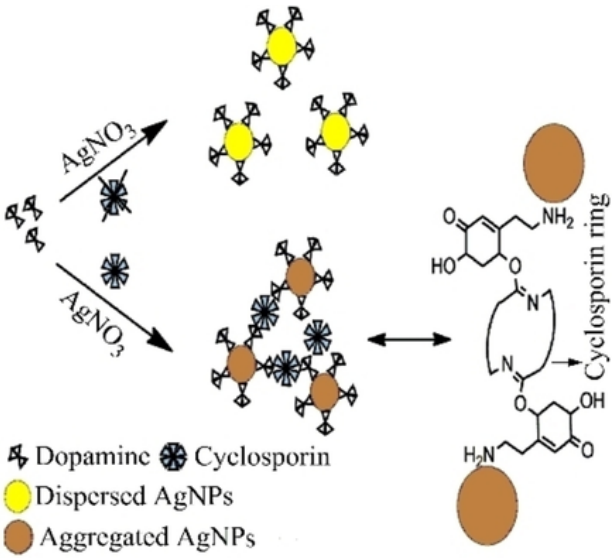


**Figure 1.** The Schematic shown of detection of CsA with colorimetric AgNPs based probe reported by Jahed et al with reprinting license code of 5217170346639.[12]

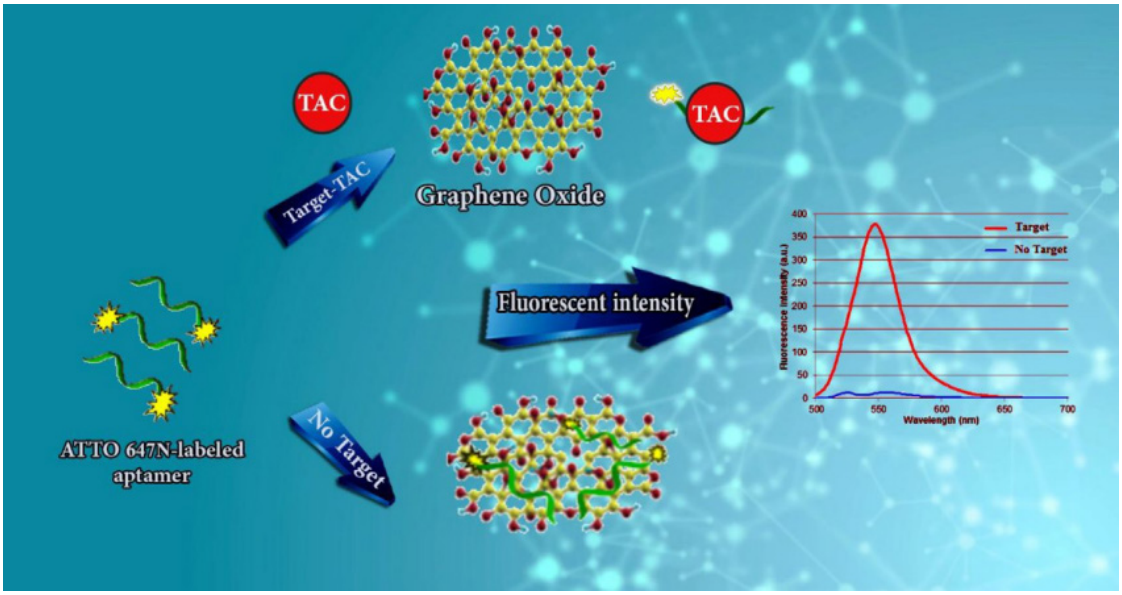


**Figure 2.** The Schematic illustration of detection of TAC via ATTO 647 N-labeled Apt122 sensor in presence of GO reported by Mansouri et al with reprinting license code of 5217170657449.[15]

technique has consisted of the enhancement of the calcein–$KMnO_4$ signal *via* AgNPs in the presence of AZT. The linear range of this method was from $5.0 \times 10^{-3}$ to $2.0 \times 10^{3}$ µM and the LOD of $2.6 \times 10^{-4}$ µM which defined no significant difference between the two methods.[13] By the way, due to the importance of fast detection methods in TDM of the drugs, it is important to apply the suggested method for biological samples.

Recently, electrospinning has been known as a cost-effective method to draw very fine polymer fibers in various diameter ranges. In fact, due to their extended specific surface area, high interconnectivity, and the ability to control the fiber diameter they can prevail over the conventional fibers limitations.[16] Rezaei et al utilized electrospun polyvinyl alcohol (PVA) nanofibers which the Ag nanoparticles decorated on it by chitosan (CS) as a polymer auxiliary. Both PVA and CS are biocompatible and nontoxic materials used as a framework for AgNPs as intensive SPR sensors to detect AZT. Moreover, they used ascorbic acid on the surface of the probe, which, if reduced, decreases the AgNPs formation on the probe surface, and consequently reduces the intensity of plasmon resonance of AgNPs. The developed probe demonstrates a linear response from 0.14 to 2.88 µM AZT concentration with the LOD of 0.09 µM and it was applied to AZT quantification in pharmaceutical formulation and human serum samples.[17]

Electrochemical methods have been considered due to the utilities of simple operation, cost-effectiveness, high durability, portability, and speedy response. Santy et al employed an electrochemical sensor to detect the electro-oxidation of MMF via pencil graphite electrode (PGE). PGE is one of the most promising electrode materials in drug analysis researches. Obtained results from cyclic voltammetry (CV) demonstrated that there are two irreversible oxidation peaks at 0.66 V and 0.84 V and the differential pulse voltammetry observations exhibit two dynamic ranges 0.02-0.3 µM and 0.3-1 µM for MMF determination by the developed sensor. Meanwhile, the LOD of the proposed sensor was as low as $1.8 \times 10^{-3}$ µM. In addition, the sensor has an appropriate selectivity for MMF determination in pharmaceutical formulation and urine samples.[18] In another study by Prashanth et al, the reduction of graphene oxide was used as an electrochemical strategy for the quantification of MMF. The reduced graphene oxide film was placed on a glassy carbon electrode (GCE) which lead to enhancement of the electro-oxidation peaks of MMF at 0.84 V and 1.1 V. A DPV results showed the dynamic range from 0.04 to 15 µM and LOD was calculated around $11.3 \times 10^{-3}$ µM in bulk samples and pharmaceutical formulations.[19] Another modification on GCE was reported via Shahrokhian and Ghalkhani for the determination of AZT. They used a film of nanodiamond–graphite/chitosan on the surface of GCE. Among carbon-based nanostructures in electrochemical fields, diamond has been considered as an appropriate material in contrast by traditional counterpart. The advantages include stability of the structure in anodic and cathodic potentials and excellent corrosion resistance which cause excellent long-term durability. As mentioned before, chitosan was used here for its film-forming ability. The CV results demonstrated that the modified electrode has a considerable catalytic role that not only decreases the reduction overpotential but also enhances the cathodic peak current. The developed electrode exhibited a dynamic response from 0.2 to 100 µM of AZT concentrations and the LOD was computed about $65 \times 10^{-3}$ µM. Also, it was applied to the quantification of trace amounts of AZT in pharmaceutical dosage form and human serum samples.[20]

As an electrochemical detection of AZT, Dehdari Vais et al designed the sensor by electroposition of pyramidal nanoparticles on the gold surface in the presence of lysine. The voltammetric results were demonstrated the electroreduction of AZT on the gold surface and the DPV

measurements exhibited a linear dynamic range from 0.095 to 900 µM with LOD of 0.090 µM. The suggested method was applied for AZT determination in tablet formulation which should be tested in biological samples in further studies.[21]

Nowadays, the combination of transition metal/metal oxide-based and carbon materials to developed sensing platforms has been attracted the interest in electrochemistry field. In other literature, an electrochemical platform by decorating screen-printed carbon electrode modified by manganese oxide/reduced graphene oxide was reported for AZT determination via Selvi et al. In comparison with other electrodes, the fabricated one has an extended surface area to transfer electrons which cause the high conductive behavior and effective detection of AZT. Differential pulse voltammetry results evaluated a dynamic range from 9 to 573.5 × $10^3$ µM with a low LOD of 4 ×$10^{-3}$ µM. Also, the sensor showed acceptable with the relative standard deviation value of about 4.96% and 4.89%. In the end, the fabricated sensor was applied to use AZT determination in human blood serum and urine samples.[9]

In a different study, Zhang et al developed an electrochemical immunosensor for the determination of TAC. The development of the sensor was based on a spherical carrier amplification strategy in which the conjugation of gold nanorods (AuNRs) functionalized L-cysteine onto polystyrene (PS) nanoparticles was prepared as a spherical signal carrier. This structure has a wide surface for loading capture antibodies. The structure of the synthesized biosensor was illustrated in detail in Figure 3. The labeling of PS, as a stable spherical functional polymer, with AuNRs@L-Cys improves the conductivity of the designed sensor, and then the PS-AuNRs@L-Cys structure was linked to capture antibodies via glutaraldehyde. In the end, a single layer of molybdenum disulfide ($MoS_2$) which was fixed *via* chitosan was added to enhance the carrying capacity and stability of the electrode. This immunosensor revealed a linear relationship for TAC concentration in the range of 1.0 × $10^{-3}$ to 3 × $10^{-2}$ µM and LOD of 1.7 × $10^{-3}$ µM. Finally, the developed immunosensor was used for the determination of TAC concentrations in human serum samples. [22]

Both optical and electrochemical-based sensors are considered as a fast analysis method with low-cost easy miniaturized which can be utilized for in-situ monitoring in POC applications. However, these methods need more validation processes to achieve a reliable sensor that has comparable sensitivity and selectivity with traditional methods. Also, as an important factor for using these

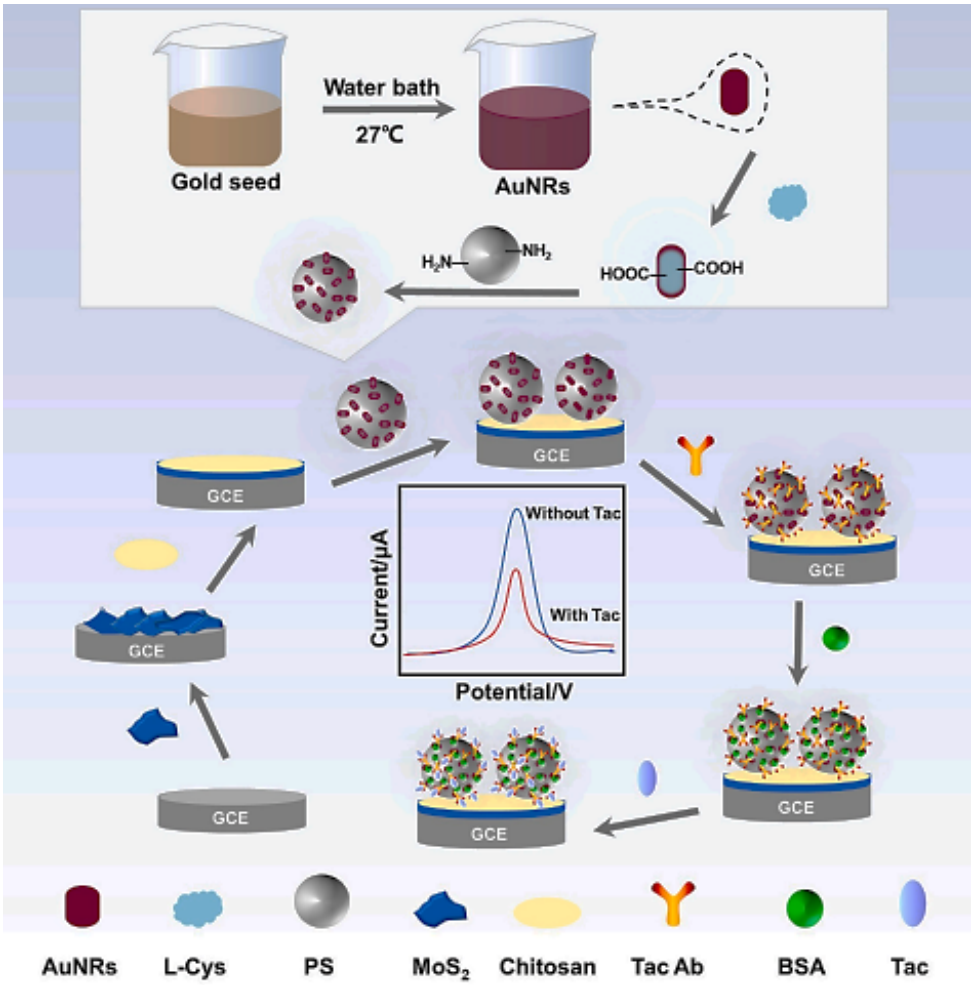


**Figure 3.** The Schematic illustration of the synthesis process of polystyrene-gold nanorods @L-cysteine/$MoS_2$ for detection of TAC reported by Zhang et al with reprinting license code of 5217170835110.[22]

methods for TDM, their function should be applied in the determination of drugs in biological samples. Even more, in the study reported by Jahed et al the performance of the developed sensor for CsA detection was tested in the patients following oral administration which made the proposed method more reliable than others. In the end, further researches are needed to develop novel methods for fast detection which have more simple operation, sensitivity and selectivity to use as a POC device.

## Conclusion

The recently reported methods for calcineurin inhibitors and purine synthesis inhibitor agents non-enzymatic detection are reviewed. Each of the reported sensors has its own beneficial features and limitations. Optical and electrochemical techniques are dominating in the detection of the drug while electrochemical methods were more applied. Electrochemical methods have been considered due to the utilities of simple operation, cost-effectiveness, high durability, portability, and speedy response. It seems that the detection of these types of pharmaceuticals has not been widely implemented using the fast detection method and may be regarded as a potent field for designing future analytical methods.

### Acknowledgments

The author would like to acknowledge the supports from Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

### Authors' Contribution

ZG and AJ: Writing original draft; JS: Reviewing; AJ: Conceptualization and approval of the manuscript

### Funding Sources

Not applicable.

### Ethics Issues

None.

### Conflict of Interest

None.

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