Analytica Chimica Acta xxx (2017) 1–9

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

# Competitive amperometric immunosensor for determination of p53 protein in urine with carbon nanotubes/gold nanoparticles screen-printed electrodes: A potential rapid and noninvasive screening tool for early diagnosis of urinary tract carcinoma

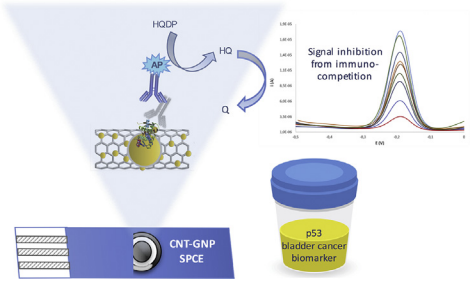
Marco Giannetto*, Maria Vittoria Bianchi, Monica Mattarozzi, Maria Careri

*Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy*

## HIGHLIGHTS

- First competitive amperometric immunosensor for determination of p53 in urine.
- High selectivity, trueness and precision associated to easy preparation and low-cost.
- Performance comparable to high-cost sensors based on complex nanostructured architectures.
- Diagnostic tool valuable for screening and follow-up programs in urologic malignancies.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 12 March 2017
Received in revised form
10 August 2017
Accepted 6 September 2017
Available online xxx

*Keywords:*
Competitive immunosensor
p53 protein
Urine
Bladder carcinoma

## ABSTRACT

Since p53 protein has become recognized biomarker for both diagnostic and therapeutic purposes in oncological diseases with particular relevance for bladder cancer, it is highly desirable to search for a novel sensing tool for detecting the patient's p53 level at the early stage. Here we report the first study on the development and validation of a novel disposable competitive amperometric immunosensor for determination of p53 protein at subnanomolar levels, based on p53 immobilization on gold nanoparticles/carbon nanotubes modified screen-printed carbon electrodes. The assay protocol requires the use of single anti-p53 mouse monoclonal antibody (DO-7 clone), able to recognize both wild-type and mutant p53. The developed immunosensor as well as the protocol of the electrochemical immunoassay were optimized by means of an experimental design procedure to assess the suitability of the device to be validated and applied for the determination of p53 in untreated and undiluted urine samples. It was found that the developed competitive immunodevice was able to achieve wide linear range detection of wild-type p53 from 20 pM to 10 nM with a low detection limit of 14 pM in synthetic urine samples, suggesting the sensor's capability of working in a complex sample matrix. The excellent performance results also in terms of selectivity, trueness and precision, coupled with the advantages of an easy preparation and low-cost assay in contrast to other methods which require very complex, time-consuming and costly nanostructured architectures, makes the developed competitive immunosensor an analytically robust diagnostic tool, valuable for implementation of screening and follow-up programs in patients with urologic malignancies.



* Corresponding author.
*E-mail address:* marco.giannetto@unipr.it (M. Giannetto).

http://dx.doi.org/10.1016/j.aca.2017.09.005
0003-2670/

## 1. Introduction

p53 is an effective transcription factor, responding to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism [1]. The majority of the carcinogenic mechanisms involve mutations of such a protein [2,3], the mutant p53, characterized by longer half-life than its wild-type counterpart, being frequently (over)expressed in extracellular physiological fluids, such as blood or urine, from patients with clinically conclamated cancer, as well as from some subjects exposed to genotoxic and mutagenic agents. As a consequence, p53 attends a key defense role against cancer inception and progression, acting as a "guardian of the genome", being currently considered as a biomarker of crucial importance for both diagnostic and therapeutic purposes [4].

Cancer protein biomarkers are specifically related to oncologic diseases, and their levels in physiological fluids or body tissues can provide information valuable for clinical screening programs, as well as early cancer detection, with significant advantages in terms of costs for societies and healthcare system. Consequently, the need for reliable analytical approaches for tumor biomarkers determination at early stage of carcinogenic diseases assumes a paramount importance.

Bladder Transitional Cell Carcinoma (BTCC), one of the most common malignancies in urology, is the fourth most common cancer in men and the eighth in women [5]. Also for BTCC, the findings of molecular genetic studies evidenced the main role played by the mutations in the suppressor genes, responsible for the pathogenic mechanism of the malignancy. p53 is one of these suppressor genes, especially occurring in bladder tumors [6], with very short half-life (20–60 min) in its wild-type form, reaching up to 6 h [7] upon certain structural mutations. Several clinical studies demonstrated that abnormal p53 protein accumulation in the epithelial cells of the whole urinary tract may result in its overexpression at level of extracellular fluids, with particular relevance for urine [8–11], indicating p53 as a valuable protein biomarker associated to pathological and clinical parameters of the disease.

On the basis of the increasing clinical interest for the determination of p53 in cell lysates and biological fluids, a variety of analytical approaches, encompassing matrix-assisted laser desorption/ionization time-of-flight mass spectrometry combined to high-performance liquid chromatography pre-fractioning [12], immunochemical methods [13,14] and surface-enhanced Raman spectroscopy [15] have been developed. Concerning sensing devices field effect transistors [16] as well as surface plasmon resonators [17] have been investigated as biologically addressable substrates for the selective determination of p53. A recently published review paper [18] provides an overview of the various types of immunosensors and immunoassay developed for the determination of p53, highlighting also technological advancements over the last several years in response to the need of new, easy-to-use, home and decentralized diagnostics of cancer markers as p53. In particular, amperometric immunosensors are very versatile tools for rapid and user-friendly screening analysis of biomarkers of clinical concern [19], thanks to their compatibility with portable and compact instrumentation, exploiting disposable screen-printed electrodes as sensing substrates [20,21]. These devices match the fundamental needs required for the screening analysis of biological samples in terms of reliability, simplicity of operation and of the instrumentation, fast time and cost-effectiveness, associated to good sensitivity and high specificity assured by targeted antibodies.

To date, there are a limited number of publications showing amperometric immunosensors for determination of p53 in samples of clinical interest. All these immunodevices are based on the working principle of the sandwich Enzime Linked ImmunoSorbent Assay (ELISA), involving the use of a capture antibody, properly immobilized as bioreceptor on the electrode surface and paired with a secondary antibody able to recognize a different epitope of the target antigen. In most cases the secondary antibody is not available as conjugated with the labeling enzyme, requiring the use of a third enzyme-labeled reading anti-antibody. A graphene-based immunosensor for electrochemical quantification of p53 phosphorylated on serine 15 (phospho-p53$^{15}$), based on sandwich immunoassay involving a phospho-p53 capture antibody, a biotinylated phospho-p53$^{15}$ detection antibody and horseradish peroxidase (HRP)-labeled streptavidin was developed by Xie et al. [2]. In the same year, other researchers devised a new electrochemical immunosensor aimed at ultrasensitive detection of phosphorylated p53 at serine 392 (phospho-p53$^{392}$) based on graphene oxide (GO) as nanocarrier in a multienzyme amplification strategy [22]. Again, phospho-p53$^{392}$ was the target of a nanomaterial enhanced disposable immunosensor using enzyme functionalization of carbon nanospheres as a signal amplification label and magnetic beads coupled with screen-printed carbon electrodes as electrochemical transducers [23].

Concerning immunosensors of unphosphorylated p53, more recently the target protein was sandwiched between biotinylated capture anti-p53 immobilized on gold nanoparticles/graphene oxide electrode surface *via* thiolated GO/streptavidin-Au NPs and secondary anti-p53 labeled with HRP, realizing a complex but ultrasensitive electrochemical immunosensor [24].

However, to the best of our knowledge, there is no published research addressing the development of competitive approaches for the immunochemical determination of p53, neither by colorimetric ELISA nor by electrochemical immunosensors. In such a context, this is the first report on the development and validation of a novel disposable competitive amperometric immunosensor for determination of p53 at subnanomolar levels, based on gold nanoparticles/carbon nanotubes modified screen-printed carbon electrodes (CNT/GNP SPCE) directly functionalized with p53 protein. The assay protocol requires the use of single anti-p53 mouse monoclonal antibody (DO-7 clone), able to recognize both wild-type and mutant p53. The developed competitive immunosensor foreshadows as a simple, reliable and analytically robust diagnostic tool, valuable for implementation of screening and follow-up programs in patients with urological malignancies. Moreover, the functionalization of the device with the target antigen, exploited for competitive approach could be also suited for determination of anti-p53 antibodies on the same sensing substrate, by easy-to-perform sample incubation, without immunocompetition.

The combination of gold nanoparticles and single-walled carbon nanotubes in the screen-printed electrode substrate was exploited for enhanced sensitivity of the immunosensor thanks to the high active surface for the immobilization of p53 on nanogold particles, in association with enhancement of the electron transfer rate in the diffusive detection of the hydroquinone (HQ) enzymatically produced upon dephosphorylation of non-electroactive hydroquinone diphosphate (HQDP). The immunosorbed anti-p53 antibodies are in fact detected through Alkaline Phosphatase-conjugated secondary Rabbit Anti-Mouse antibodies (RAM-AP). The developed immunosensor as well as the protocol of the electrochemical immunoassay were optimized by means of an experimental design procedure to assess the suitability of the device to be validated and applied for the determination of p53 in clinical samples (untreated

Please cite this article in press as: M. Giannetto, et al., Competitive amperometric immunosensor for determination of p53 protein in urine with carbon nanotubes/gold nanoparticles screen-printed electrodes: A potential rapid and noninvasive screening tool for early diagnosis of urinary tract carcinoma, Analytica Chimica Acta (2017), http://dx.doi.org/10.1016/j.aca.2017.09.005