



# Recent Electrochemical Methods Proposed for the Detection of Hepatitis C Virus. A Minireview

RUXANDRA-MARIA ILIE-MIHAI<sup>1\*</sup>  <https://orcid.org/0000-0002-2285-9583>

RALUCA-IOANA STEFAN-VAN STADEN<sup>1\*</sup>  <https://orcid.org/0000-0001-8244-2483>

JACOBUS (KOOS) FREDERICK VAN STADEN<sup>1</sup>  <https://orcid.org/0000-0002-9684-005X>

HASSAN Y. ABOUL-ENEIN<sup>2</sup>

<sup>1</sup>Laboratory of Electrochemistry and PATLAB, National Institute of Research for Electrochemistry and Condensed Matter, 202, Splaiul Independentei Str., Bucharest-6, Romania

<sup>2</sup>Pharmaceutical and Medicinal Chemistry Department, The Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Cairo 12311, Egypt

**Abstract:** *Hepatitis C virus is very common amongst population infected with viral hepatitis. A very important point before deciding on the correct treatment is the stage of illness. More people are detected too late, and therefore the treatment was not efficient. A highly sensitive and selective screening system for early detection is needed in order to cure the patient. Therefore, this review is summarizing new methods of detection of specific biomarkers/genotypes for viral hepatitis C, to help find better sensitive screening methods that may influence the decision of the most efficient treatment.*

**Keywords:** *hepatitis C, biomedical analysis, sensors, electrochemical detection*

## Introduction

Hepatitis C virus is a major global health issue, and a major cause of liver diseases [1]. Worldwide it is estimated that 130–170 million people are chronically infected with HCV, with 3–4 million new infections per year. In Europe the highest prevalence of HCV infections is in the IDU population but most carriers are unaware of their infection status (<http://www.who.int/mediacentre/factsheets/fs164/en/>).

The most important risk factor for HCV infection is past or current injection drug utilization, with most studies reporting a prevalence of 50% or more [2]. HCV is a member of the genus Hepacivirus within the Flaviviridae family and contains a linear single-stranded RNA genome of 9600 nucleotides in length. The HCV genome encodes a long polyprotein precursor of more than 3000 amino acids. This polyprotein is processed into ten mature proteins by host and viral proteases at the endoplasmic reticulum (ER), including structural (Core, E1, E2) and non-structural (p7, NS2 to NS5B) proteins [3]. HCV infection can cause fibrosis, liver cirrhosis and hepatocellular carcinoma [4, 5].

To date there are six major genotypes of HCV each with multiple subtypes, which have been identified worldwide [6]. Genotype 1 is the predominant genotype in many geographical regions, including Europe and North America, where it accounts for 50–90% of the cases. Genotype 2 is relatively common in Europe, North America and Japan and Genotype 3 is common in Southeast Asia, Australia, South America and northern Europe, in particular in intravenous drug users. Genotype 4 is mainly found in Egypt, the Middle East and central Africa, genotype 5 in southern Africa and genotype 6 in south-east Asia [7]. The genotype dictates the chance of therapeutic response and duration of treatment for patients with hepatitis C. Genotypes 2 and 3 respond favorably (75% sustained response, SR) on 24 weeks of treatment with pegylated interferon and ribavirin, while genotype 1 responds worse (50% SR) even after 48 weeks of treatment. The suitable treatment regimen for genotypes 4–6 is not yet established [8].

\*email: [i.ruxandra04@yahoo.com](mailto:i.ruxandra04@yahoo.com), [ralucavanstaden@gmail.com](mailto:ralucavanstaden@gmail.com)

Many studies unequivocally demonstrated the association between the HCV genotype and the responsiveness to antiviral combination therapy with pegylated interferon alpha and ribavirin [9] making HCV genotyping an indispensable tool for the tailoring of antiviral treatment and the diagnostic follow-up of the patients. Genotype 1 is the most common in the US and also the type least responsive to treatment [10].

Being a member of the flaviviridae family, hepatitis c virus (hcv) is an infectious agent, provoking liver deterioration, succeeded by cell destruction [11]. A significant deterioration of the liver is caused, on one hand by the inflammation spread throughout the body as a response to fighting the virus, and on the other hand by the virus. If infected with hepatitis C, some people may develop symptoms, while others remain asymptomatic. Sooner or later, a chronic infection will be developed in both cases. The deterioration inflicted by the inflammation is enough to distress the liver, but not enough to destroy the virus completely.

Asymptomatic people may develop cirrhosis of liver, and some may develop even hepatocellular carcinoma (HCC); even those with specific symptoms can manifest cirrhosis or HCC if they delay the medical visit [12]. Chronic hepatitis C (CHC) inflicts numerous complications, with lethal consequences for patients, cases in which cirrhosis is present in 20% of cases, and HCC with an incident of 4-5% in a year [13]. Epidemiologists implied that hepatitis C virus is connected with other medical conditions, which are not related with the hepatic manifestations, counting glomerulopathies, insulin resistance, oral expressions and type 2 diabetes [14-16].

The diagnosis of a disease, especially a viral one, is to analyze and care for infected persons; that being its principal concern. Also, it represents one of the ways of how to stop virus dispersion and evolution of disease. In general, it is hard to say when the primary HCV infection has taken place, because there are no precisely indicators, and the infected persons do not know due to lack of symptoms.

HCV infection is usually diagnosed during evaluation of an incidental finding of abnormal liver function or during screening of high-risk populations with serologic assays for specific anti-HCV antibody or by polymerase chain reaction (PCR) molecular assays for HCV RNA viral load [17].

Pegylated interferon in combination with ribavirin has been universally adopted universally for therapy of chronic HCV infection [18-21] for 24–48 weeks, and the response to therapy depending on HCV genotype [22]. Therapy is expensive and shorter courses of treatment result in significant savings. Many patients cannot benefit from the current treatment regimen (interferon/ribavirin combinations) due to its side effects or ineffectiveness.

Liver biopsy is the standard solution for defining the severity of liver disease. Testing for viral antigens, among which hepatitis C virus (HCV) is established in clinical virology. This strategy has been hampered by difficulties in expressing surface antigens and to produce monoclonal or polyclonal antibodies able to detect these antigens [23].

Discovery and development of effective antiviral agents to combat hepatitis C virus (HCV) is the focus of intensive research both in academic environment and in pharmaceutical companies. One of the HCV nonstructural proteins, NS5B, represents an attractive target in light of the clinical success of human immunodeficiency virus reverse transcriptase inhibitors [24].

HCV has been detected in saliva, semen, and other nonserological fluids of some plasma-positive patients [25], now virological studies have been undertaken to determine whether HCV is present in the nasal secretions of drug users, a necessary precondition for internasal viral transmission. James McMahon and collaborators [26] reported in their work, preliminary findings on the detection of HCV RNA in the nasal secretions of plasma-positive chronic drug sniffers.

For clinical practice can be used four HCV markers, total anti-HCV antibodies, the HCV core antigen levels, HCV RNA levels and the HCV genotypes.

Early detection is the key to prevent chronic infection therefore, earlier detection of infection results in earlier treatment and thus earlier recovery. A rapid and accurate diagnosis of HCV is important for the prevention of viral transmission and management of disease progression. For this reason, there is

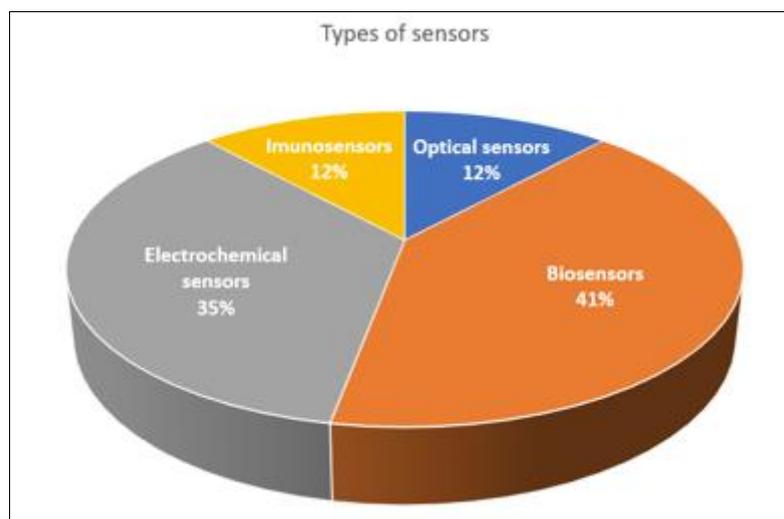
still a great need for a tool to simplify the detection of HCV with acceptable sensitivity and specificity, short turnaround time, and cost-effectiveness.

There are a number of diagnostic tests for hepatitis C virus infection including detection of anti-HCV antibody and quantification of HCV RNA [27, 28]. Screening of antibodies against HCV is not a reliable method of diagnosing acute HCV infection. This method cannot distinguish between a current or past infection because people will retain anti-HCV antibodies for life once they are exposed to HCV. In addition, testing anti-HCV antibody might provide false negative or positive results because it takes more time (45–68 days) to develop anti-HCV antibody post HCV infection [29]. It is also expensive and labor-intensive for routine use. However, screening for HCV RNA is currently regarded as the method of choice for the confirmation of an active infection in both immunocompetent patients who are anti-HCV positive and immunocompromised individuals who may not mount an adequate antibody response [30].

In 1999 was developed the first assay for HCV core antigen (HCV Ag) [31] and several others followed in the next few years [32-34], and the attention was focused on the core gene products which have been demonstrated to circulate in the bloodstream of infected individuals [35]. It has been reported that HCV core antigen can be detected in the serum for most patients during the acute infection [36, 37].

HCV core antigen levels correlate well with HCV RNA levels and may consequently be an indirect marker of HCV replication as a low-cost alternative for diagnosis of HCV acute infection [38 - 40]. Given the generally favorable performance characteristics of HCV core antigen assays, it seemed reasonable to further refine this analytical format [28, 41].

### Electrochemical methods used for the diagnostic of Hepatitis C virus



**Figure 1.** Assignment of the electrochemical sensors used for the analysis of HCV virus and antigen (the figure is the concept of the authors of this review)

The electrochemical immunosensors, based on the highly specific molecular recognition of antigen by its antibodies, have developed a high interest in clinical diagnostics and will be expected to provide fast and highly sensitive detection of HCV. However, there is less report use of the electrochemical immunosensor for detection of HCV antigen. It is valuable to explore the possibility to use electrochemical sensors to detect HCV antigens for its clinical application. The advantages of these devices are excellent sensitivity and selectivity, rapid response and cost-effectiveness of the nanostructured materials choice to design of the biosensor or sensor for screening of the HCV antigen (Figure 1).

Nanotechnology and nanoscience shows new opportunities for design of electrochemical sensors, various nanomaterials have been utilized for construction of these devices due to their physical and chemical properties, namely: high mechanical strength, wealthy electronic and catalytic properties, electrical conductivity, large and high surface area, excellent biocompatibility and good chemical stability [42 - 44]. Nanostructured materials have been explored to design a new generation of electronic and electrochemical tools for pattern recognition of various biological compounds through electrical and electrochemical signals. These electrical and electrochemical responses are based generally on the changes in the ohmic response of an electrical circuit or the flow of electrons arising from faradaic processes (i.e., oxidation or reduction) near the surface of an electrode [45, 46].

Quantification of HCV antigen in biological fluids, human serum and whole blood, in incipient stage, is essential and may be used as an indicator for the early diagnosis, disease progression, personalized treatment and monitoring of therapy for patients infected with this virus [28], so the development of highly sensitive and selective devices for the screening of this antigen is a challenging task.

### Immunosensors

Immunosensors and sensors are capable to detect selectively target analytes (HCV) at a very low concentration (Table 1). Some reports have already been published on electrochemical sensing/screening of hepatitis B virus [47-50] but, despite considerable interest in developing simple and reliable methods for detection and quantification of HCV, there are few works for HCV detection based on electrochemical techniques (Table 1) [51,52].

**Table 1.** Detection of HCV using electrochemical techniques

Biomarker/antigen	Electrode	Method	Concentration range	LOD	References
HCV core antigen	AuNPs/SiO <sub>2</sub> -Chit nanocomposite integrate with secondary antibody (Ab <sub>2</sub> ) without labeled HRP.	Cyclic voltammetry and electrochemical impedance spectroscopy	2 - 512 ng mL <sup>-1</sup>	0.17 ng mL <sup>-1</sup>	Ma [53]
HCV core antigen	MultisHRP-DNA-coated CMWNTs modified with GMCs-MB nanocomposite and Au nanoparticles.	Square wave voltammetry	0.25-300 pg mL <sup>-1</sup>	lowest of 0.01 pg mL <sup>-1</sup>	Ma [54]
HCV	HCV antibody immobilized on nanogold particles and protein A.	Potentiometry	1.5-350 ng mL <sup>-1</sup>	0.8 ng mL <sup>-1</sup>	Tang [55]
HCV	3-Mercaptopropionic acid assembled on gold electrode, activated by 1[3(dimethylamino)propyl]-3-ethylcarbodiimide methyl iodide (EDC) and N-hydroxysuccinimide (NHS) thionine and the HCV horseradish peroxidase (HRP) antibody.	Linear scan voltammetry	3.2-16 mg L <sup>-1</sup>	-	Minjian [56]
HCV non-structural 5A protein.	Glassy carbon electrode modified with an Au MoO <sub>3</sub> /Chitosan nanocomposite.	Chronoamperometry	1 ng mL <sup>-1</sup> - 50 µg mL <sup>-1</sup>	lowest of 1 ng mL <sup>-1</sup>	Liang [57]
HCV NS3-4A protease	A self-assembled monolayer of a ferrocene amino acid derivative and a short NS3-4A specific inhibitory peptide (Asp-Glu-Ile-Val-Pro-Nva) were attached to the electrode surface.	Electrochemical techniques	10 – 100pmol L <sup>-1</sup>	5 pmol L <sup>-1</sup>	Sowole [58]
HCV core antigen	MWCNTs-Chit/GCE	Cyclic voltammetry, differential pulse voltammetry, electrochemical impedance spectroscopy	5.0fg mL <sup>-1</sup> - 1.0pg mL <sup>-1</sup>	1.67fg mL <sup>-1</sup>	Ghanbari [59]



HCV DNA	Cucurbit[7]uril(CB) and nucleic acid exonuclease III functionalized with graphene oxide	Square wave voltammetry	0.2-10nmol L <sup>-1</sup>	160.4pmol L <sup>-1</sup>	Jiang [60]
HCV virus	Graphene oxide with ethylenediamine compound immobilized on the outer layer of the gold sensor	Differential pulse voltammetry and electrochemical impedance spectroscopy	-	1.36nmol L <sup>-1</sup>	Oliveira [61]
HCV core antigen	Three types of chitosan mixed with gold nanoparticles and diamond paste	Chronoamperometry	40fg mL <sup>-1</sup> – 4ng mL <sup>-1</sup>	40fg mL <sup>-1</sup>	Moldoveanu [62]
Hepatitis C 3a virus	Gold electrode modified with a self-assembled monolayer composed of a peptide nucleic acid (PNA) probe and 6-mercapto-1-hexanol.	Electrochemical techniques	-	5.7 x 10 <sup>-11</sup> mol L <sup>-1</sup>	Hejazi [63]
HCV virus	Screen-printed electrode functionalized with cystamine (C4H12N2S2) and glutaraldehyde (C5H8O2) to make the covalent binding with the recombinant core-HCV protein	Cyclic voltammetry	-	1ng μL <sup>-1</sup>	Costa [64]
HCV virus	Polyaniline functionalized on metallic organic framework	Electrochemical impedance spectroscopy	1.0fmol L <sup>-1</sup> – 100nmol L <sup>-1</sup>	0.75fm L <sup>-1</sup>	Sheta [65]
HCV core antigen	HCV core antigen—with a nanowire aptasensor	Electron beam lithography	-	0.3pg mL <sup>-1</sup>	Malsagova [66]
HCV-RNA	Silver/zinc bimetallic metallic organic framework	Differential pulse voltammetry	1fmol L <sup>-1</sup> – 100nmol L <sup>-1</sup>	0.64fmol L <sup>-1</sup>	El-Sheikh [67]
HCV core antigen	Glassy carbon electrode modified with graphene quantum dots	Electrochemical impedance spectroscopy	10-70 and 70-400pg mL <sup>-1</sup>	3.3 pg mL <sup>-1</sup>	Ghanbari [68]
HCV core antigen	Deoxynucleotidyl transferase (TdT) amplification and DNA nanowires (DNW)	Differential pulse voltammetry	0.1-312.5pg mL <sup>-1</sup>	32fg mL <sup>-1</sup>	Wang [69]

Cuixia Ma and collaborators [53] developed in 2012 a novel and reliable procedure to construct label-free electrochemical immunosensor for the detection of HCV core antigen with a sandwich immunosensor based on AuNPs/ZrO<sub>2</sub>-Chit nanocomposites which demonstrated excellent biocompatibility and electrochemical behavior when AuNPs were directly synthesized on the surface of ZrO<sub>2</sub> nanoparticles in one-step procedure using chitosan as reducing agent. Anti-HCV was immobilized on this nanocomposite to fabricate the immunosensor. The secondary antibody was immobilized on AuNPs/SiO<sub>2</sub>-Chits nanocomposite. Under optimized conditions, the immunosensor exhibited board liner range between 2 and 512 ng mL<sup>-1</sup>, good stability and high sensitivity for the detection of HCV core antigen, and with a detection limit of 0.17 ng mL<sup>-1</sup>.

An ultrasensitive and selective electrochemical immunosensor was developed [54] for the detection of hepatitis C virus (HCV) core antigen recently. The design of the immunosensor is based on graphitized mesoporous carbon–methylene blue (GMCs–MB) nanocomposite as an electrode modified material and a horseradish peroxidase-DNA-coated carboxyl multi-wall carbon nanotubes (CMWNTs) as a secondary antibody layer. Under optimum conditions, the amperometric signal increased linearly with the core antigen concentration (0.25 pg mL<sup>-1</sup> to 300 pg mL<sup>-1</sup>). The detection limit of the sensor was low as 0.01 pg mL<sup>-1</sup> and it has a high selectivity. The new protocol showed acceptable stability and reproducibility, as well as favorable recovery for HCV core antigen in human serum. The proposed immunosensor has great potential for clinical applications.

An incorporated programmed electrochemical immunosensor [55] exhibit has been intended for the synchronous identification of 5-type hepatitis antigens (for example hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E). At first, 5-type hepatitis antibodies were immobilized onto an independent electrochemical sensor cluster utilizing nanogold particles and protein A as frameworks; the relating agents were caught by a one-step capture format by the immunosensor from sample solution. The identification depends on the likely change when the antigen-antibody response by utilizing a 2-cathode framework. The created immunosensor exhibit with permitted concurrent assurance of 5-type hepatitis antigens in 5 min. For most analytes, the lowest detected limit was ≤1.0 ng/mL. The created



immunoassay framework offers guarantee for mark free, straightforward, fast, financially savvy examination of multi-analyte. Critically, the chip-based immunosensor could open new doors for high-throughput multi-analyte immunoassays in purpose in care testing of mini- lab-on-a-chip gadgets.

Another method [56] on the detection of Hepatitis C infection (HCV) using an electrochemical immunosensor was accounted for. 3-Mercaptopropionic acid was collected on gold electrode to shape a self-gather monolayer. After the changed electrode was initiated by 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide methiodide (EDC) and N-hydroxysuccinimide (NHS), thionine and horseradish peroxidase (HRP) immunizer were covalently limited to oneself gather monolayer, to shape the Hepatitis C electrochemical immunosensor. Cyclic voltammetry and direct sweep voltammetry were utilized to explore the gathering system and the compound qualities of the immunosensor. The concentration range of Hepatitis C was represented in the domain of 3.2-16 mg/L with a correlation coefficient of 0.995 was gotten by LSV. While utilized in the clinical utilization of human serum, this strategy was in great accordance with ELISA.

### Electrochemical sensors

A glassy carbon electrode modified with an Au-MoO<sub>3</sub>/Chitosan nanocomposite (justify a good conductivity and biocompatibility of this electrode), has been used [57] for the molecular recognition of the hepatitis C virus non-structural 5A protein. The electrochemical response has been obtained as an amperometric i-t curve (chronoamperometry). This electrochemical electrode based on nanostructured materials cover the linear concentration range from 1 ng mL<sup>-1</sup> to 50 µg mL<sup>-1</sup>, and the detection limit has been as low as 1 ng mL<sup>-1</sup>. The electrochemical electrode showed excellent reproducibility, high selectivity, and a good stability for the molecular recognition of hepatitis C virus non-structural 5A protein. It was successfully applied to the detection of the antigen in real serum samples.

The detection of hepatitis C viral NS3-4A protease has been possible [58] using a self-assembled monolayer of a ferrocene amino acid derivative and a short NS3-4A specific inhibitory peptide (Asp-Glu-Ile-Val-Pro-Nva) attached to the surface of the working electrode. The interaction of the peptide was quantified using electrochemical techniques in the linear concentration range of 10 - 100 pM with a detection limit of 5 pM.

In the carbon domain, carbon multi wall nanotubes are considered one of the best platform on which you can lay or attached numerous molecules and proteins. In his study, Ghanbari et al [59], evolved the growth of CWNT platforms, by introducing the union of molecular imprinting process with aptasensors, for hepatitis antigen core detection. They started from a glassy carbon electrode which they modified with carbon wall nanotubes and chitosan. CWNT were chosen due to their peculiar and unique properties, and chitosan for its high characteristics of adhesions and that the hydroxyl and amine groups makes chitosan receptive to chemical alterations. A hepatitis core antigen complex was fixed on the nanocomposite MWCNTs-Chit/GCE and further exposed to 13 patterns of dopamine electro-polymerization. The high dependability and straightforwardness of nanocomposite based stage combined with awareness and selectivity of the hybrid receptors, additionally profiting from the twofold explicit atomic acknowledgment property of MIPs and Apt is one of the benefits of this strategy. The insightful presentation and application plausibility of the sensor was estimated by a few different electrochemical strategies, for example, cyclic voltammetric (CV), differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS). The sensor response demonstrated that the construction method was successful; the results obtained by utilizing this modified sensor had a detection limit of 1.67 fg/mL, and a concentration range from 5.0 fg/mL to 1.0 pg/mL. Likewise, the sensor was additionally inspected to perceive the protein explicitly in human serum to examine its true capacity in clinical utilization. Notwithstanding their precision and dependability, this large number of approaches has specific pragmatic disadvantages like significant expense and unwieldy application. Execution of the sensor was better than that of different strategies announced in different journals, particularly in contrast with the detailed LODs. One more benefit is the lower cost of the proposed strategy. Overlay, this proposed



technique can be effectively stretched out and can possibly be applied for distinguishing different antigens in serum tests.

Jiang and his collaborators [60], present the idea of a host-guest interconnection which takes place amongst cucurbit[7]uril(CB) and nucleic acid exonuclease III, for a sensitive identification of HCV DNA. The CB was blended by UV crosslinking with graphene oxide functionalized together with azide, with further developed sensitivity of electrochemical recognition. Considering the host-guest collaboration among CB[7] and MB, the CB[7] atoms caught the methylene blue (MB) particles in the arrangement, advanced the electron-transport among MBs and anode surfaces. GO went about as an awesome vector for the insertion of CB. Accepting this as a sign model, an exceptionally touchy identification technique for HCV DNA was accounted for by utilizing acid exonuclease III, which has amazing exodeoxyribonuclease movement on twofold abandoned DNA with blunt finishes from 3' to 5' end. In order to validate the creation of CB[7]-N3-GO, an electrochemical technique and TEM were used for characterization of the final sensor. The quantitative analysis of HCV DNA probe was realized using the CB[7]-N3-GO sensor and the square wave voltammetry technique. The results obtained proved that the detection strategy present a wide detection domain within the range of 0.2-10 nmol/L, and a detection limit as low as 160.4 pmol/L. In the interim, the proposed methodology can separate the subtype of HCV DNAs, and also it exhibits a forthcoming potential in the blood sample screening for HCV in clinical investigation.

A new study was presented by Oliveira and her research team [61], study in which they altered graphene oxide with ethylenediamine compound for the HCV DNA test immobilization on the outer layer of the gold sensor, pointing the development of another demonstrative stage for the electrochemical recognition of hepatitis C infection genomic RNA.

The shift that takes place among graphene oxide and gold electrode, followed by an ETD chemical alteration, interceded by the utilization of EDC/NHS, was proficient for the immobilization of the HCV1 test; the steps of the sensor's constructions were approved by the morphological and electrochemical examinations. The outcomes acquired by the EIS are in line with the voltammetric examinations, and are further validated by the enhancement of the oxidation current or resistance in each phase of alteration of the gold sensor and acknowledgment of the HCVgRNA. In accordance, the SEM and AFM results reveal a critical change in morphology and rugosity when hybridization of the particular target takes place. The created electrochemical genosensor was explicit and particular in recognizing HCVgRNA, confined in human serum from various contaminated patients, making an interpretation of this biorecognition occasion into a quantifiable sign in just 20 min of test, with an identification cutoff of  $1.36 \text{ nmol L}^{-1}$  of RNA. These outcomes are promising in the advancement of a new genosensor for the finding of hepatitis C in clinical examination.

Another research study that choose chitosan as a modifier for its electrochemical sensors, is the study introduced by Moldoveanu et al [62], for a fast and precise detection of Hepatitis C virus core antigen. In their academic work, the researchers selected three types of chitosan, based on their molecular weight, owing to their various but essential characteristics, such as good biocompatibility, good adhesion, biodegradability, its antimicrobial effect. Nanomaterials are of great importance for constructing electrochemical sensors because increases the sensitivity of the sensor. Therefore, they developed three stochastic microsensors based on nanomaterials such as gold and diamond powder, and chitosan as modifier solution. The three sensors were constructed starting from diamond powder mixed with paraffin oil, then altered with a solution of gold nanoparticles and three types of chitosan, which were differentiated by their molecular weight. A chronoamperometric method was selected in order to test and validate the sensors. The stochastic sensors response relies on channel conductivity. There are two stages presented, the first stage gives the analyte its distinctive signature ( $t_{\text{off}}$ ) and is a qualitative parameter, and the second stage where redox processes take place, give the quantitative parameter. The qualitative parameter is also useful due to the fact that it give the sensors characteristics, such as the linear concentration range that was between 40 fg/mL and 4 ng/mL, the detection limit or the sensitivity.

Among the three sensors, the one that had the best response, with high sensitivity and detected the lowest concentration of hepatitis c core antigen was chitosan III/AuNps/DP. The validation of the sensors was proven by assaying whole blood samples for detection of the analyte, with recovery values bigger than 98%. It is safe to say that these sensors have a great future in the electrochemical sensor domain; they have shown that a fast, selective and cost-effectively method can be employed in diagnosis of biological samples of HCV core antigen.

## Biosensors

Development of an electrochemical DNA biosensor, using a gold electrode modified with a self-assembled monolayer composed of a peptide nucleic acid (PNA) probe and 6-mercapto-1-hexanol was described by Hejazi and his coauthors [63]. The biosensor was used for electrochemical detection of short sequences of hepatitis C 3a virus. The limit of detection was  $5.7 \times 10^{-11}$  M with a relative standard deviation of 1.4% in phosphate buffer solution, pH 7.0.

Moreover, due to the fact that laboratory equipment is expensive and requires qualified personnel, Costa et al [64], were motivated to develop a low-cost system, namely a portable laboratory platform (PLP) that can realize electrochemical reactions and also the manufacturing of amperometric biosensors. The system was formed on a hardware-software design, so that cyclic voltammetry (CV) would be performed in the same manner as it would be performed on a commercialized potentiostat. One of its applications could be used in immunochemical diagnosis, thus implying low costs and a reduction of analysis time, currently addressed in the diagnosis of HCV. The constructed PLP contains several parts, such as a controller unit, a potentiostat circuit able to perform CV and a Bluetooth module, enabling smartphones contact. The Bluetooth module is needed so that the parameters for the measurements can be defined on the smartphone.

A subtractive procedure was enhanced to manufacture the electrochemical sensors, by utilizing a mechanism called printed circuit board (PCB) and the conductive surface of the sensors was altered through an electroplating and electroless process. A fine film of copper and fibre glass fulfilled the role as substrate of the sensors, by deposition process. For completing the three-electrode system, the counter and working electrode were amended with gold and the reference electrode with silver. The final form of the biosensor was achieved by following a few steps: the first step was the cystamine functionalization of the sensor, the second step is represented by the activation of the surface with glutaraldehyde, followed by the third step, immobilization on the surface of the sensor of hepatitis C antigen, and the last step is antibody- antigen interaction (seizing the HCV antibody). The functionalization of the biosensor was realized in order to obtain a covalent binding with the recombinant core-HCV protein. The developed system provided reliable results by using CV technique. The validation of the entire structure was achieved through immunodiagnostic of hepatitis C, structure that re-united functionalized biosensors, SAM (self-assembled monolayers) and a covalent bond between the antigen-antibody complex, which in return favoured high stability and reproducibility, and a comparable detection limit of antibody with a value of  $1 \text{ ng} \cdot \mu\text{L}^{-1}$ .

On the other hand, Sheta et al [65] constructed an innovative electrochemical biosensor employing a metal-organic framework (MOF) decorated with polyaniline and nickel, for a straightforward determination of HCV nucleic acid. The base of the biosensor is represented by a glassy carbon electrode, on which the polyaniline nanocomposite was coated using layer-by-layer deposition, followed by treatment with DNA probe and bovine serum albumin. After the treatment, the new robust biosensor exhibited good electrical conductivity and high porosity. These features of the sensor were validated after the connection made between the HCV probe and target, which led to the formation of the nucleic acid double strands, and to an enhanced resistance against charge transfer. The raw materials used in this research together with the on-site formed materials were depicted utilizing a variety of characterization techniques, both analytically and spectroscopically, such as XRD, XPS, mass-spectrometry, UV-VIS/NIR, elemental analysis, SEM/EDX, atomic absorption spectroscopy, FT-IR etc.

Electrochemical strategies, cyclic voltammetry and electrochemical impedance spectroscopy were utilized to screen the effective manufacture of the proposed biosensor and identification of the ability of recognition of the target. The outcomes of the procedure and of the biosensor were of great value, evidencing that the sensor is sensible enough to identify the HCV antigen even when nonspecific nucleic acids are present; a low limit of detection of 0.75 fM being obtained, completed by a linear range comprising from 1 fM to 100 nM.

Moreover, Malsagova et al [66], demonstrated that the core antigen of HCV, also known as the protein marker, can be detected in real-time, using a nanowire biosensor. The concept of the sensor is made out of a p-type conductance chip, and on its surface were laid structures which form silicon-on-insulator (SOI) nanowires. The late kind have been manufactured through electron beam lithography and gas-plasma treatment. Surface of the sensor was sensitized by adding some aptamers against hepatitis core antigen, thus proofing the specificity of the sensor's detection ability. A great influence had the phosphate buffer concerning the sensor. It was observed that a change was registered, when the pH changed from acid to neutral, which had an effect on the invert polarity of the sensor's signal. A low limit of detection was obtained in both acidic and neutral buffer solution, yielding a value of ~10-15 M, using the SOI-NW-based aptasensor. The study also showed that a good reproducibility was obtained when repeated determinations of hepatitis core antigen took place in both serum samples of hepatitis C patients and buffer solutions.

Another work that make use of metal organic framework, is the research conducted by El-Sheikh et al [67]. The authors of this study, produced a MOF decorated with silver and zinc by reflux method. They further used characterization equipment, FT-IR, UV-VIS/NIR, mass spectrometry, XRD, SEM/EDX to analyse the compound. In order to obtain the final version of the sensor, first, the bimetallic MOF was shed upon a glassy carbon electrode. Second, the hepatitis core probe was immobilized onto the surface of the electrode by adsorption. In the last part, the electrode was treated with bovine serum albumin (BSA), to hinder all active sites that can be found on the surface of the electrode. For a better demonstration that the fabrication of the final version of the sensor was successfully obtained, two electrochemical methods were utilized, electrochemical impedance spectroscopy and cyclic voltammetry. Furthermore, another electrochemical technique was applied, differential pulse voltammetry (DPV), in the presence of glucose, to confirm that bimetallic MOF's catalytic activity is the trigger through which gluconolactone is obtained, by oxidation of glucose. The result is represented by an increase in electrochemical signal of the electrode. Also, the utilization of DPV technique was to investigate the specificity and sensitivity of the sensor. The exhibited hepatitis biosensor in this work, showed a couple of characteristics that are essential in the sensor category, such as ease to use, high values of sensitivities and sensibilities, and by its utilization it was possible to obtain a very low detection limit (0.64 fM) and also a wide detection domain from 1 fM to 100 nM.

For the first time, Ghanbari et al [68], fabricated an uncomplicated aptasensor for a ultra-sensitive assay of hepatitis C antigen. The construction of the aptasensor started from the foundation, a glassy carbon electrode modified with graphene quantum dots. GQDs were employed for the immobilization phase of the sensor's surface, task that was fulfilled through  $\pi$ - $\pi$  interactions and non-covalent interactions of hydrogen bonds. This special type of carbon nanoparticles were chosen due to their significant electrochemical and chemical characteristics, like their unique size and surface effect. For detection of the HCV antigen core, a redox probe was inaugurated in the form of ferrocyanide. The authors resorted to electrochemical techniques, like cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy to validate the modifications that took place at the surface of the aptasensor which were performed through a series of chemical modifications. For detection of hepatitis C core antigen, the research showed that by using EIS as an alternative method of detection and in conjunction with the presented aptasensor it was possible to detect the antigen at pico level, to be precisely the lowest limit of determination was  $3.3 \text{ pg mL}^{-1}$ , with two concentration ranges that varied between  $10\text{-}70 \text{ pg mL}^{-1}$  and  $70\text{-}400 \text{ pg mL}^{-1}$ . In addition, the study was taken to another level, by assaying

human serum samples in order to detect the hepatitis antigen. It was proved that the created aptasensor could validly detect the antigen from biological sample. The verdict of this study is that the GQD nanocomposite and the chosen method presented numerous benefits, a prompt, sensitive and selective path for HCV antigen detection, with low limit of detection and a wide domain of concentration, and also a low cost of fabrication of the sensor and easy to use.

A new and modern electrochemical biosensor was accomplished by Wang et al [69]. Together with his team, they started to construct the biosensor by adding a few drops of HCV Antibodies combined with BSA to form the antibody-antigen recognition, followed by an antibody-conjugated DNA that was further drawn to the surface of the GC and spread into a DNA sequence of considerable length, called DNA nanowires, by a deoxynucleotidyl transferase (TdT) reaction. To these nanowires, a substantial amount of methylene blue-loaded DNW (MB@DNW) were attached, to stand as signal labels. The HCV antigen probe, perceived both the first antibody that was fixed on the surface of the electrode and also the second antibody that was linked to the DNA. At the 3' end of the DNA, through deoxynucleotidyl transferase amplification, was connected a poly(T) sequence, the underling layer used was thymidine. The outcomes are represented by an increase of the DPV signal, the biosensor indicating a good reliability and a detection limit of femtogram magnitude order. The results obtained, compared with other studies, indicate better results, both for the limit of detection that had a value of 32 fg/mL and a wider concentration range, from 0.1 to 312.5 pg/mL, a good example of comparing being the result acquired from ELISA kit, 5 pg/mL. The hybridization of the DNA antigen that was performed with the methylene blue-DNA nanowires generated an enlarged signal for the identification of HVC antigen. All the more significantly, by correlation with ELISA and a good recovery test, it was made possible the identification of HCVcAg from biological samples by using the proposed biosensor. In this way, the created electrochemical technique offers a possible device for an exact and sensitive identification of HCVcAg.

### **Optical methods methods used for the diagnostic of Hepatitis C virus**

In the last couple of years there were several academic studies (Table 2) that investigated whole-cell antigen procedures, so the investigations continued to present time. Venkatesh et al [70] is one of the group of researchers that carried on the studies. They demonstrated both clinically and from the development point of view, that were able to create an immunosensor starting from reagents that releases yeasts, binded through non-covalently bonds to an electrochemical circuit. They started from two approaches, one being yeast proteins excretion, and the second one being the discharge of the surface-protein.

The first approach was taken in consideration, but soon after, the researchers concluded that because of disulfide interference, the hepatitis proteins are deprived of their antigenicity. Therefore, they reoriented to the second approach, where it was shown that the yeast proteins cling to the gold surface of an electrode and of direct antibodies, leading to a higher yielding. For validation of yeast excretion, a fluorescent and immunochemical studies were conducted.

So, they continued on second path, by adding the excreted proteins and yeast supernatant on the surface of a gold overlay electrode. The choice of using yeast excretion can be explained by the incorporation of solid consistency of fermentation with the post-translational activity of eukaryotes, eliminating at the same time supplementary purification-only moieties. In the end, they were successful in developing Hepatitis C virus (HCV)-core antigen- GBP fusion proteins. The manufactured immunosensors were assayed for cross-reactivity and their capacity *in vitro*. They exhibited good stability and selectivity and distinctive attachment to mono- and polyclonal antibodies *in vitro* tests, limit of detection of 32 nM. The final test, was to prove the validity of the HCV antigen immunosensors, by assaying clinical isolates. A defined differentiation was found between the infected and non-infected patients, the type of sample tested was human serum. Therefore, it was clearly demonstrated that the platform constructed has the ability to discern between positive and negative HCV biological samples.

Over the years, technology has become worldwide available to all humans, regardless of their status. It is not surprisingly to see that smartphones have become valuable tools in the research domain. Teengam and his team [71], is one of the research teams that involved the use of technology, especially smartphones, in the process of detecting biomarkers in different types of diseases. In their study, Teengam et al, announced a novel point-of-care system, namely a paper-based DNA sensor type, that includes a fluorescence method of detection for HCV DNA assaying. The starting point of development, involved a wax-printing strategy for manufacturing the paper-based sensor. Further, pyrrolidinyl peptide nucleic acid (acpcPNA) probe was covalently attached onto the detection zone of the cellulose sensor, by reduction alkylation reaction between aldehyde and amine groups. The attachment was performed with an ssDNA-specific fluorescent dye that helps visualizing the DNA target coupled to the nucleic acid, with no need of a futuristic probe design or a dye-quencher pair. The nucleic acid probe was a compulsory need, due to the fact that it exhibits no response when the dye was involved. The researchers also constructed a movable gear that reunites a source of light, for the fluorescence excitation, and a camera that is able to detect the fluorescent emission that came from a smartphone, which plays the role of optical-readout unit. The camera was efficient in detecting the fluorescence signal response that came from the selective binding between the fluorescent dye and the single-strand region of the DNA target; the signal response was also perceived by naked eye. When the conditions of the experiment were optimised, the cellulose sensor indicated that among the HCV DNA and the green channel a fluorescent change is observed, conducting to the following experimental results: a concentration range between 5 to 100 pmol with a correlation coefficient of 0.9956, and a limit of detection of 5 pmol. Prominently, the detection limit was upgraded when the length of the shade district of the DNA target was expanded and, in this manner, the created framework was acceptably applied for the discovery of HCV cDNA from clinical examples without requiring PCR or different sorts of DNA intensification. Profiting from the sign intensification accomplished through the various restricting destinations for the color given by the overhanging tail of long ssDNA target groupings, this framework was effectively applied to distinguish the HCV corresponding DNA (cDNA) got from clinical examples with acceptable outcomes. The proposed fluorescent paper-based sensor exhibited an extraordinary potential to be utilized as a minimal expense, basic, mark free, touchy, and specific DNA sensor for point-of-care applications.

**Table 2.** Detection of HCV using optical techniques

Biomarker/antigen	Electrode	Method	Concentration range	LOD	References
HCV virus	Hepatitis C virus (HCV)-core antigen- gold-binding peptide	Fluorescent immunoassay	32 nmol L <sup>-1</sup> – 3.2 μmol L <sup>-1</sup>	32nmol L <sup>-1</sup>	Venkatesh [70]
HCV virus	Pyrrolidinyl peptide nucleic acid (acpcPNA) covalently attached onto paper-based sensor	Fluorescent detection	5-100pmol L <sup>-1</sup>	5pmol L <sup>-1</sup>	Teengam [71]

## Conclusions

Although there are medicines to treat efficiently the patients with hepatitis C virus, if the detection of the virus was not done at an early stage or as early as possible after the symptoms appeared, the chances to cure are decreasing. Furthermore, viral hepatitis may favorize the development of tumoral cells.

At this stage of development, the standard methods are not sensitive enough to determine very early the presence of specific biomarkers of viral hepatitis C in whole blood, saliva, and other biological fluids. The researchers are concentrated more on developing new sensitive methods for viral hepatitis B, and less to hepatitis C - which is more common among the population. Therefore, it is a need to develop these methods, and to adapt the treatment to the stage of illness.



**Acknowledgments:** This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number PN-III-P4-ID-PCE-2020-0059, within PNCDI III.

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Manuscript received: 8.03.2022