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GILVÂNIA MARINETE DE SANTANA

BIOSSENSORES ELETROQUÍMICOS BASEADOS EM FILMES COMPÓSITOS DE NANOMATERIAIS DE CARBONO PARA DIAGNÓSTICO MOLÉCULAR E SOROLOGICO DA HEPATITE C

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2019

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas da Universidade Federal de Pernambuco, como requisito parcial para a obtenção do título de Doutor em Ciências Biológicas.

Área de concentração: Biotecnologia.

Orientadora: Prof[a]. Dr[a]. Rosa Amália Fireman Dutra

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Aprovada em: 11/03/2019

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Aos meus pais **Paulo e Marinete,** aos meus irmãos **Valdilene, Lucivânia, Givanildo, Leonaldo e Walterlins** e aos meus sobrinhos **Sther, Julia, Miguel e Maria Luiza**, por todo amor, carinho e incentivo.

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RESUMO

A infecção pelo vírus da hepatite C (HCV) atinge cerca de 170 milhões de pessoas no mundo, sendo um grave problema de saúde pública. Devido à doença ser, em muitos casos, assintomática levando a cronificação, podendo levar à cirrose e carcinoma hepatocelular, a disponibilidade de testes práticos e rápidos que auxiliem no diagnóstico é desejável. Entre os métodos de diagnóstico, o desenvolvimento de nanocompósitos resultantes de polímeros e materiais de carbono tem auxiliado no preparo de superfícies sensoras, proporcionando um aumento na transferência eletrônica e maior sensibilidade analítica aos biossensores. O diagnóstico do HCV é realizado através de imunoensaios na pesquisa de anticorpos contra HCV (anti-HCV), que se confirmados positivamente, necessitam de testes moleculares para detectar o cDNA-HCV. Nessa tese, foram desenvolvidos três sensores eletroquímicos à base de filmes nanoestruturados de carbono; o primeiro, utilizando oxido de grafeno (GO) e polipirrol (PPy), o segundo, GO e politiofeno (PTh), e o terceiro, nanotubos de carbono (NTC) e poli-L-lisina (PLL) para a detecção de anti-HCV e RNA viral, respectivamente. A primeira plataforma nanoestruturada foi obtida por eletropolimerização usando-se voltametria cíclica, e apresentou uma excelente estabilidade eletroquímica (Coeficiente de Variação (CV) ≅ 0,75%). A técnica de voltametria de onda quadrada foi usada para obtenção das respostas analíticas, observando-se detecção de Anti-HCV numa faixa linear de 2 a 10 $ng.mL^{-1}$ ($r = 0.995$, $p < 0.01$). Na segunda plataforma, o nanocompósito de GO e PTh, foi sintetizado em uma única etapa utilizando-se a técnica de voltametria cíclica e brometo de cetramonio (CTAB) como solvente. A plataforma demostrou-se estável (CV ≅ 0,6), verificada através da técnica de voltametria cíclica, e específica para o diagnóstico do HCV (LOD = 0.07 $ng\ mL^{-1}$), verificada pela técnica de onde quadrada. No terceiro, utilizou-se a técnica de *dropcasting* para deposição de filmes de NTCs sobre a superfície de PLL, obtida por eletropolimerização. Uma sequência conservada de 20 pares de base (pb), de RNA-HCV, foi utilizada como sonda para hibridização do RNA complementar do HCV (cDNA). Foi possível discriminar amostras positivas em soro enriquecido com as fitas complementares.

Palavras-Chaves: Nanotubo de Carbono. Poli-L-lisina. Grafeno. Polipirrol. Politiofeno. Hepatite C.

ABSTRACT

Hepatitis C virus (HCV) infection affects about 170 million people worldwide and is a serious public health problem. The main form of transmission is parenteral, and less often sexual and vertical. Because the disease is often asymptomatic and has a high chance of chronicity, leading to cirrhosis and hepatocellular carcinoma, the availability of rapid and practical tests to aid in the diagnosis, dissemination and worsening of the disease is desirable. Among the diagnostic methods, the electrochemical biosensors can meet the demands mentioned, since they involve simple technologies, measuring biomolecular interactions through electrical parameters, be it current variation, potential, etc. In the last decade, the development of nanocomposites resulting from polymers and carbon materials has assisted in the preparation of sensorial surfaces, providing greater analytical sensitivity to biosensors. The diagnosis of HCV is performed through immunoassays in the detection of antibodies against HCV (anti-HCV), which if confirmed positively, require molecular tests to detect viral RNA. In this thesis, three electrochemical sensors were developed based on nanostructured carbon films; the first, using graphene oxide (GO) and polypyrrole (PPy), the second, GO and polythiophene (PTh), and the third, carbon nanotubes (NTC) and poly-L-lysine (PLL) for the detection of anti -HCV and viral RNA, respectively. The association of carbon allotropes to polymer films has allowed greater stability to the sensor matrices, which may result in an increase in electronic transfer. The first nanostructured platform was obtained by electropolymerization using cyclic voltammetry, and presented excellent electrochemical stability (Coefficient of Variation (CV) ≅ 0.75%). The square-wave voltammetry technique was used to obtain the analytical responses, with detection of Anti-HCV in a linear range of 2 to 10 ng.mL^{-1} ($r = 0.995$, $p < 0.01$). In the second platform, the nanocomposite of GO and PTh was synthesized in a single step using the technique of cyclic voltammetry and cetrammonium bromide (CTAB) as solvent. The platform was shown to be stable (CV ≅ 0.6), verified by the cyclic voltammetry technique, and specific for the diagnosis of HCV (LOD = 0.07 ng mL^{-1}), verified by the square technique. In the third, the dropcasting technique was used to deposit NTC films on the PLL surface obtained by electropolymerization. A conserved sequence of 20 base pairs (bp) was used as a probe for hybridization of complementary HCV RNA.

Keywords: Carbon Nanotube. Poly-L-lysine. Graphene. Polypyrrole. Polythiophene. Hepatitis C.

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LISTA DE ABREVIATURAS E SIGLAS

CNT *Nanotubos de Carbono, do inglês Carbon Nanotubes*

CV *Voltametria Cíclica, do inglês Cyclic Voltammetry*

CV *Coeficiente de Variação*

CTAB *Brometo de Cetrimônio, do inglês Cetrimonium bromide*

DNA *Ácido Desoxirribonucleico, do inglês Deoxyribonucleic Acid*

GCE *Eletrodo de Carbono Vítreo, do inglês Glassy Carbon Electrode*

GO *Oxido de Grafeno, do inglês Graphene Oxide*

HBV *Vírus da Hepatite B, do inglês Hepatitis B Virus*

HCV *Vírus da Hepatite C, do inglês Hepatitis C vírus*

HIV *Vírus da Imunodeficiência humana, do inglês human immunodeficiency vírus*

IpA *Pico anódico*

IpC *Pico Catódico*

IUPAC *União Internacional de Química Pura e Aplicada do inglês International Union of Pure and Applied Chemistry*

OMS *Organização Mundial da Saúde*

PLL *Poli-l-lisina, do inglês Poly-L-Lysin*

POCT *Do inglês point-of-care testing*

PPy *Polipirrol, do inglês Polypyrrole*

PTh *Politiofeno, do inglês Polythiophene*

PCR *Reação em Cadeia da Polimerase, do inglês polymerasechainreaction*

RNA *Ácido Ribonucleico, do inglês Ribonucleic Acid*

SA *Estrepitavidinal, do inglês streptavidin*

SWV *Voltametria de onda quadrada, do inglês Square Wave Voltammetry*

WHO *Organização Mundial da Saúde, do inglês World Health Organization*

LISTA DE SÍMBOLOS E UNIDADES

nm	*Nanômetro*
mm	*Milímetro*
mL	*Mililitro*
mmol.L^{-1}	*Milimol*
mol.L^{-1}	*Mol*
Vs-^{1}	*Voltes por segundo*
pmol	*Picomol*
µA	*Microampère*
µg	*Microgramas*

SUMÁRIO

1 INTRODUÇÃO

O vírus da Hepatite C (HCV) afeta cerca de 170 milhões de pessoas em todo o mundo. De 2000 a 2015, foram identificados no Brasil 61.297 óbitos associados às hepatites virais, destes, 1,7% foram associados à hepatite viral A; 21,6% à hepatite B; 1,1% à hepatite D e 75,6% à hepatite C (BRASIL, 2017). Rotineiramente, o diagnóstico do HCV é realizado por meio da detecção direta dos anticorpos produzidos contra proteínas virais antigênicas (estruturais e não-estruturais) no soro ou no plasma humano. Confirmados os resultados para o Anti-HCV, é necessário a realização de testes moleculares para detecção do RNA viral.

Os testes sorológicos, realizados por imunoensaios enzimáticos ou por eletroquimioluminescência, e os testes moleculares, baseados na **reação em cadeia da polimerase (PCR),** têm sido bem estabelecidos na rotina laboratorial para diagnóstico da hepatite C. Entretanto, esses necessitam de processamento em laboratório, requerendo profissionais treinados, instrumentação sofisticada, diversas etapas de processamento, além de não serem práticos para utilização em larga escala para triagem em unidades de pronto atendimento (“point-of-care”) (DROSTE, 2017).

Nas últimas décadas, os biossensores vêm sendo estudados como um dos métodos analíticos mais promissores para aplicação na área de diagnóstico laboratorial. Biossensores são dispositivos bioanalíticos, compostos por um componente biológico, como elemento de reconhecimento do analito de interesse, associado a um componente transdutor, responsável por converter o sinal bioquímico em um sinal elétrico quantificável (GAUDIN, 2017). De acordo com o tipo de sinal mensurado, os biossensores podem ser classificados como ópticos, piezelétricos e eletroquímicos. Dentre estes, os biossensores eletroquímicos destacam-se na área de desenvolvimento de testes para pronto atendimento, devido a sua praticidade, rapidez de resposta, sensibilidade e compatibilidade com as tecnologias de miniaturização de dispositivos. No diagnóstico do HCV, o monitoramento eletroquímico de interações antígeno-anticorpo (imunossensores) é utilizado para a detecção da infecção na fase crônica. Já na fase aguda da infecção, a detecção de hibridizações entre sequências de ácidos nucléicos virais (genossensores) é aplicada para determinação o tempo da infecção e tratamento mais adequado (WANG et al, 2017).

Recentemente, a contribuição dos nanomateriais na construção de biossensores eletroquímicos tem ganhado destaque, sobretudo com a utilização dos alótropos de carbono,

dentre eles ressaltam-se o óxido de grafeno (GO) e os nanotubos de carbono (NTCs) (WANG et al., 2016). O GO consiste em uma monocamada plana de átomos de carbono organizados em uma rede bidimensional (2D), sintetizado a partir do oxido de grafite, um método de baixo custo. Embora bastante utilizados em sensores eletroquímicos para o aumento da área superficial, a presença de óxidos em sua superfície (grupos carboxílicos) limita a transferência de elétrons sobre as bordas. A incorporação a polímeros condutores surge como uma estratégia de melhoramento da transferência de elétrons, bem como auxiliar na fixação do nanomaterial na superfície sensora evitando possíveis lixiviações e tornando a plataforma mais estável. Imunossensores eletroquímicos baseados em polímeros condutores vem ganhando destaque devido ao amento na sensibilidade e seletividade como exemplos dessa classe de polímeros podemos citar o pirrol e o tiofeno como matriz em nanocompósitos, ambos possuem fácil síntese, estabilidade química e possibilidade de interação com os grupos funcionais presentes nas folhas do GO (AYDEMIR et al., 2016; WANG et al., 2019).

Outro nanomaterial de destaque na área de sensores eletroquímicos são os NTCs. Estes são cilindros longos de átomos de carbono em hibridização sp^2 unidos covalentemente que possuem extraordinárias propriedades eletrônicas, alta área superficial e versatilidade para funcionalização com grupos proteína-reativo. No entanto quando dispostos aleatoriamente sobre a superfície sensores, apresentam uma reduzida transferência elétrica (SILVA et al., 2016). Visando possibilitar a orientação dos nanotubos na superfície sensora, comumente tem se empregado o uso de polímeros com diferentes grupos reativos, como por exemplo a poli-L-lisina.

Nesta tese, optou-se por desenvolver abordagens diferentes de diagnóstico do HCV, duas delas empregando um imunossensor utilizando nanocompósito de polipirrol e óxido de grafeno e politiofeno e óxido de grafeno para quantificação de anticorpos anti-HCV. Foi desenvolvido também, um genossensor baseado em um filme nanoestruturado de Poli-L-lisina e Nanotubos de Carbono para detecção do vírus da Hepatite C.

1.1 OBJETIVOS

1.1.1 Objetivos Gerais

Desenvolver biossensores eletroquímicos baseados em filmes nanocompósitos de nanotubos de carbono e grafeno para a diagnóstico molecular e sorológico da Hepatite C.

1.2.1 Objetivos Específicos

Desenvolver plataformas sensoras empregando eletrodos modificados com Nanotubo de carbono e Poli-L-Lisina (NTC-PLL) bem como modificações com Óxido de Grafeno e Polipirrol (GO-PPy) e óxido de Grafeno e Politiofeno (GO-PTh) visando à aplicação em ensaios eletroquímicos;

Efetuar análises por técnicas eletroquímicas para caracterização do sensor;

Imobilizar a sonda sintética de DNA (ssDNA) correspondente ao HCV nas plataformas sensoras;

Imobilizar o antígeno do HCV nas plataformas sensoras;

Otimizar os parâmetros experimentais para determinação do HCV, e

Estabelecer curvas analíticas dos sensores para determinação do HCV.

2 REVISÃO DE LITERATURA

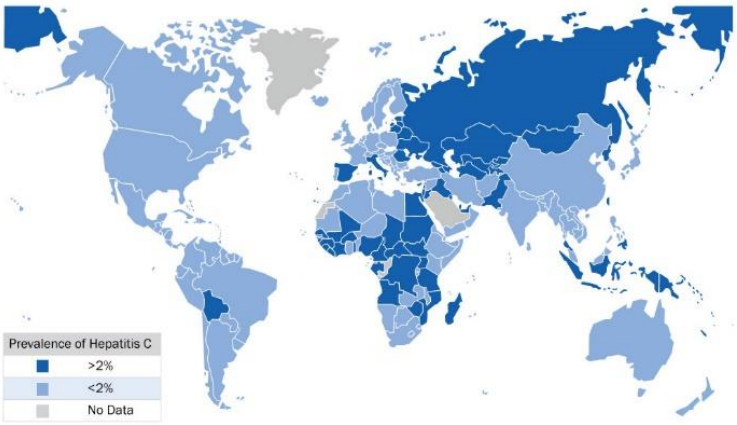
2.1 HEPATITE C: EPIDEMIOLOGIA, FISIOPATOLOGIA, TRANSMISSÃO E TRATAMENTO.

Nos últimos anos as Hepatites virais têm se tornado um grave problema de saúde. Elas estão distribuídas de maneira universal, sendo caracterizadas por serem doenças infecciosas sistemáticas que atacam o fígado, sendo a principal causa de transplante hepático do mundo. As hepatites virais são doenças provocadas por diferentes agentes etiológicos, com tropismo primário pelo tecido hepático, que apresentam características epidemiológicas, clínicas e laboratoriais semelhantes, porém, com importantes particularidades, classificam-se em seis tipos principais: A, B, C, D, E e G, dentre elas a Hepatite C se destaca, onde cerca de 85% dos indivíduos contaminados desenvolvem a forma crônica, podendo evoluir para o câncer hepático. A história natural da hepatite C é marcada por uma evolução silenciosa. Os sinais e sintomas são comuns às demais doenças parenquimatosas crônicas do fígado e costumam se manifestar apenas nas fases mais avançadas da doença. Assim, a maioria dos casos são assintomáticos e o diagnóstico da doença geralmente é tardio, ocorrendo décadas após a infecção viral. (BOSAN et al., 2010; BELYHUN et al., 2016).

De acordo com a Organização Mundial de Saúde (WHO do inglês "*World Health Organization"*) cerca de 170 milhões de pessoas estão infectadas, das quais 71 milhões possuem hepatite C crônica, o que ocasiona mais de 300 mil mortes por ano devido a complicações advindas da evolução para casos de cirrose e hepatocarcinoma (WHO, 2017). No Brasil a epidemiologia da hepatite C não é homogênea havendo grande variação regional na prevalência de cada um dos agentes etiológicos. No início da epidemia, o desconhecimento da doença e a falta de exame para o diagnóstico das infecções assintomáticas levaram à maior concentração da doença em indivíduos submetidos a transfusão de sangue e hemoderivados. As regiões em que o uso de drogas injetáveis era frequente abrigam também maior número de pessoas cronicamente infectadas pelo HCV. As hepatites virais têm grande importância pelo número de indivíduos atingidos e pela possibilidade de complicações das formas agudas e crônicas, foram notificados de 1999 a 2015 mais de 200 mil casos de hepatite C, nesse período 64,2% foram contabilizados na região Sudeste, 24,2% na região Sul, 5,6% na região Nordeste, 3,2% na região Centro-Oeste e 2,7% na região Norte (BRASIL, 2017).

A Hepatite C foi identificada em 1989, e a infecção ocorre em todos os continentes e acomete todas as classes sociais (**Figura 1**). Antes desse período apenas o "infeccioso vírus da hepatite" (vírus da hepatite A, VHA) e "vírus da hepatite sérica" (vírus da hepatite B, VHB) haviam sido identificados. No entanto, aproximadamente 65% dos casos relatados das hepatites pós-transfusionais não eram diagnosticadas como causadas pelos VHA e VHB, então esses casos foram denominados "hepatite não-A, não-B" (HNANB). Após intensa investigação, mediante sucessivos estudos de biologia molecular, CHOO e colaboradores (1989) identificaram finalmente o genoma do agente viral via clonagem molecular direta do NANB, sendo denominado de vírus da hepatite C (HCV, do inglês "*Hepatitis C Virus"*) (PESQUERO, 2010).

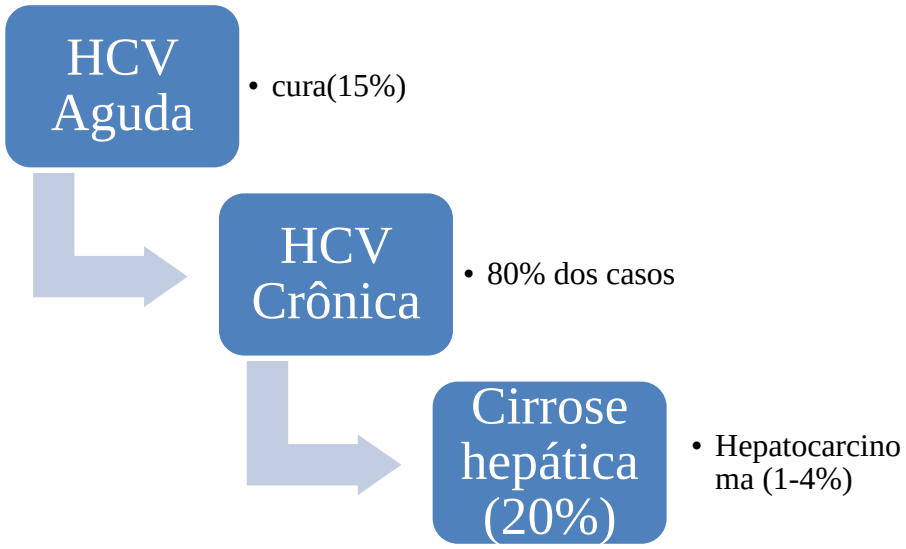
Figura 1- Mapa da incidência global da Hepatite C.



Fonte: Disponível em <https://canadiantaskforce.ca/tools-resources/hepatitis-c-clinician-faq/>. Acessado em 20/10/2017

A evolução do HCV é bastante variável, cerca de 15% a 40% dos indivíduos infectados conseguem eliminar o vírus ainda na fase aguda da doença. A maior parte dos indivíduos infectados, entretanto, evolui para a fase crônica. Destes, cerca de 20% desenvolverão cirrose hepática nos próximos 10 ou 20 anos e uma vez instalada a cirrose, os pacientes apresentam 1 a 4% de chance ao ano de desenvolver carcinoma hepatocelular (**Figura 2**) (SINGHALA, et al., 2017).

Figura 2- Fluxograma demonstrando a evolução sintomática do HCV.



Fonte: A AUTORA

Como o HCV e o Vírus da Imunodeficiência humana (HIV, do inglês "*Human Immunodeficiency Virus"*) compartilham algumas vias de transmissão, a co-infecção ocorre de maneira frequente. Outra co-infecção frequente ocorre com o Vírus da Hepatite B e com a Diabetes Mellitus. Além disso, entre 17 e 40% dos infectados com HCV apresentam manifestações extra-hepáticas comodesordens linfoproliferativas e autoimunes (DYAL et al., 2015; ABDEL-HAMEED et al., 2017).

A principal forma de transmissão do HCV é pela exposição parenteral ao sangue, objetos contaminados e produtos sanguíneos, principalmente por meio do compartilhamento de agulhas pelos usuários de drogas endovenosas, transfusão e procedimentos médicos e odontológicos, podendo também ocorrer por meio de erosões do nariz (devido à inalação de cocaína), tatuagem e "*piercing"*. A transmissão por via sexual, apesar de menos freqüente, ocorre principalmente entre em pessoas com múltiplos parceiros e com prática sexual de risco (ABREU et al., 2013).

Em geral, o período de incubação para o HCV varia de 2 semanas a 6 meses. Cerca de 80% das pessoas não apresentam sintomas após a infecção inicial, desenvolvendo geralmente a forma crônica da doença e mantendo um processo inflamatório hepático por mais de seis meses na maioria dos casos. Aqueles que são agudamente sintomáticos podem apresentar febre, fadiga, diminuição do apetite, náuseas, vômitos, dor abdominal, urina escura (colúria), fezes de cor cinza (hipocolia fecal), dor nas aticulações, icterícia, que se apresenta em cerca de 18 a 26% dos casos de hepatite aguda e inicia-se, geralmente, quando a febre desaparece.

A hepatomegalia ou hepatoesplenomegalia é também uma possível consequência da infecção por esse vírus. Todos esses sintomas são diminuídos paulatinamente (MERICAN *et. al.,* 1993; LAUER *et.al.,* 2001).

A prevenção e o controle da hepatite C dependem de uma complexa avaliação da distribuição global da infecção pelo HCV, determinação de seus fatores de risco associados e estimativa dos fatores que aceleram a progressão da doença. Além disso, devido à inexistência de uma vacina ou alguma forma de profilaxia pós-exposição, torna-se indispensável uma correta avaliação epidemiológica para o planejamento de ações de prevenção primária em qualquer população (ABDEL-HAMEED et al., 2017).

O tratamento da HCV objetiva deter a progressão da doença hepática pela inibição da replicação viral. A redução da atividade inflamatória costuma impedir a evolução para cirrose e carcinoma hepatocelular, havendo também melhora na qualidade de vida dos pacientes (SIMHA et al., 2018). Fazem parte do arsenal terapêutico para a HCV:

• Interferon (IFN) convencional alfa-2a
• IFN convencional alfa-2b
• IFN peguilado (PEG-IFN) alfa-2a
• PEG-IFN alfa-2b
• Ribavirina (RBV) 250mg

Durante o tratamento antiviral, as determinações quantitativas do HCV-RNA, por meio da cinética viral, são também fatores preditivos tanto de resposta satisfatória, como da ausência de resposta. A melhor maneira de avaliar o sucesso do tratamento é a obtenção de resposta virológica sustentada (RVS), a qual equivale a cura da infecção pelo vírus da hepatite C (HCV) e diminui a chance de evolução para cirrose, insuficiência hepática, transplante de fígado. Indivíduos com fibrose avançada, mesmo com RVS, ainda podem apresentar complicações da doença, razão pela qual devem continuar em acompanhamento médico periódico 24-26 meses (CHASCSA et al 2018).

2.2 O VÍRUS DA HEPATITE C

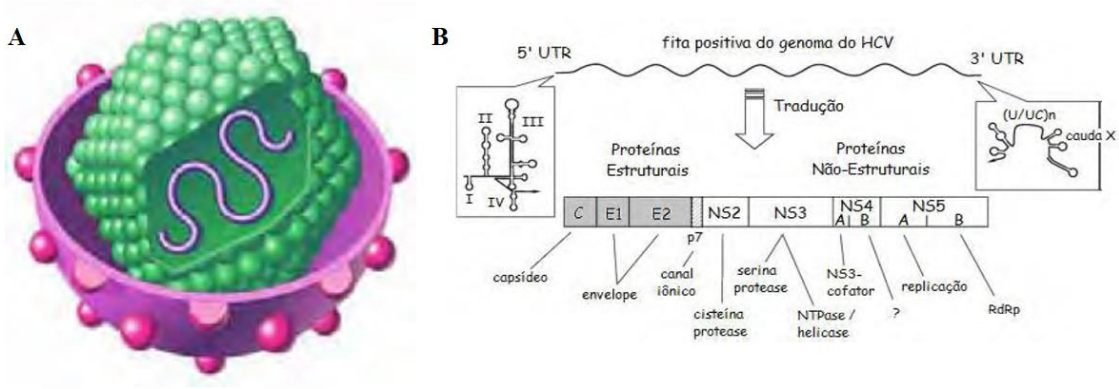
O HCV (**Figura 3A**) é um vírus de RNA pertencente à família *Flaviviridae,* do gênero *Hepacivirus,* com genoma em fita simples de polaridade positiva medindo 9,7 kilobases de comprimento, sendo o homem seu único hospedeiro natural. Esta família de vírus possui quatro tipos diferentes de géneros: flavivírus, pestivírus, hepacivírus e pegivírus. Novos

hepacivírus têm sido descritos em primatas, morcegos, carnívoros cavalos e cães, permitindo que os pesquisadores possam desenvolver novos modelos sistemas para a análise da biologia molecular e da patogênese do HCV (TANAKA et al., 2017).

A partícula viral do HCV isolado em cultura de células apresenta um envelope esférico contendo tetrâmeros (ou dímeros de heterodímeros) das glicoproteínas E1 e E2 do capsídeo, com um diâmetro de aproximadamente 55-65 nm, além disso, um nucleocapsídeo, estrutura formada pelo capsídeo e pelo genoma viral (PALAU et al., 2013). De forma semelhante a um RNA do hospedeiro, o RNA genômico do HCV serve como RNA mensageiro (mRNA) para a síntese de suas proteínas virais. O vírus possui uma estrutura linear, com uma longa sequência de leitura aberta (ORF, do inglês "*Open Reading Frame*") (**Figura 3 B**) que codifica uma poliproteína precursora com aproximadamente 3000 resíduos de aminoácidos. Durante a replicação viral a poliproteína é clivada por enzimas virais e hospedeiras em três proteínas estruturais (núcleo, E1, E2) e sete proteínas não estruturais (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B). Uma proteína adicional denominada F (frameshift ou ribossomal) ou ARF (quadro de leitura alternativo) é previsto como resultado de frameshifting, ou síntese proteica ribossomal, durante a tradução dentro da região central do genoma (ARONOFF-SPENCER et al., 2016)

Os genes que codificam as proteínas estruturais do núcleo viral e a as proteínas E1 e E2 do envelope estão localizadas no terminal 5 'da ORF, que apresenta em seguida regiões de codificação para as proteínas não-estrutural p7, NS2, NS3, NS4A, NS4B, NS5A e NS5B. As proteínas estruturais são componentes essenciais das partículas virais do HCV, considerando que as proteínas não-estruturais não estão associadas com a estrutura viral, mas são envolvidos na replicação do RNA e na morfogênese do virion (TZARUM et al., 2018).

Figura 3 - a) Representação esquemática da partícula viral e b) sequência genômica das proteínas codificadas pelo HCV.



Fonte: PESQUERO (2013).

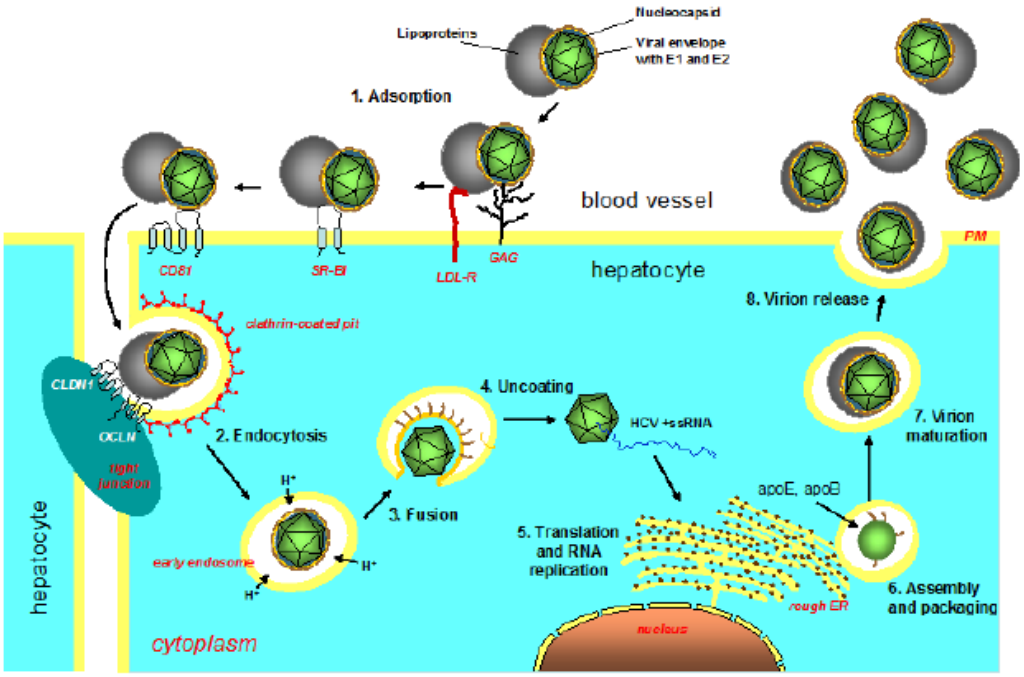
A enzima percussora do processo de replicação do HCV é a NS5B RdRP (RNA dependente RNA polimerase), ela possibilita a síntese da fita de RNA negativa por meio da fita de RNA positiva, e assim sucessivamente. A região do RNA viral pertencente à proteína NS5B é mais conservada, sendo considerada o principal alvo de intervenção antiviral (PESQUERO, 2013; GERRESHEIM et al., 2017; GEDEDZHA et al., 2017).

Análises filogenéticas das sequências genômicas do HCV, em indivíduos de diferentes regiões geográficas, permitiram a caracterização de 7 genótipos (1 a 7) que são subdivididos em grupos (a, b, c, etc.). No Brasil pode ser encontrado os genótipos 1, 2, 3, 4 e 5. As frequências gerais no Brasil são de 64,9% para o genótipo 1; 4,6% para o genótipo 2; 30,2% para o genótipo 3; 0,2% para o genótipo 4 e 0,1% para o genótipo 5. Dentro de um mesmo genótipo e subtipo podemos ainda ter variações do HCV, que são denominadas quasispecies. Isso é possível devido à replicação imperfeita do vírus, com o surgimento de pequenas e constantes mutações. A maior ou menor diversidade das quasispecies parece estar relacionada com a pressão imunológica, já que costuma ser pequena nas fases iniciais da doença, sendo de alta heterogeneidade nos casos de doença hepática mais avançada e/ou baixa resposta terapêutica (GUZ et al., 2009; GUSTAFSSON et al., 2014; NYAN & SWINSON, 2016).

Com o crescente desenvolvimento de pequenos modelos animais e sistemas de replicação do VHC in vitro mais eficientes a análise detalhada das diferentes etapas de replicação se tornou possível. Na **Figura 4** esta esquematizado o ciclo de viral da HCV, é possível notar que é necessária uma cascata de interações célula-vírus para a infecção de

hepatócitos, sendo este um mecanismo complexo e ainda não completamente compreendido (FOSTER et al., 2016).

Figura 4 - Ciclo de vida da HCV



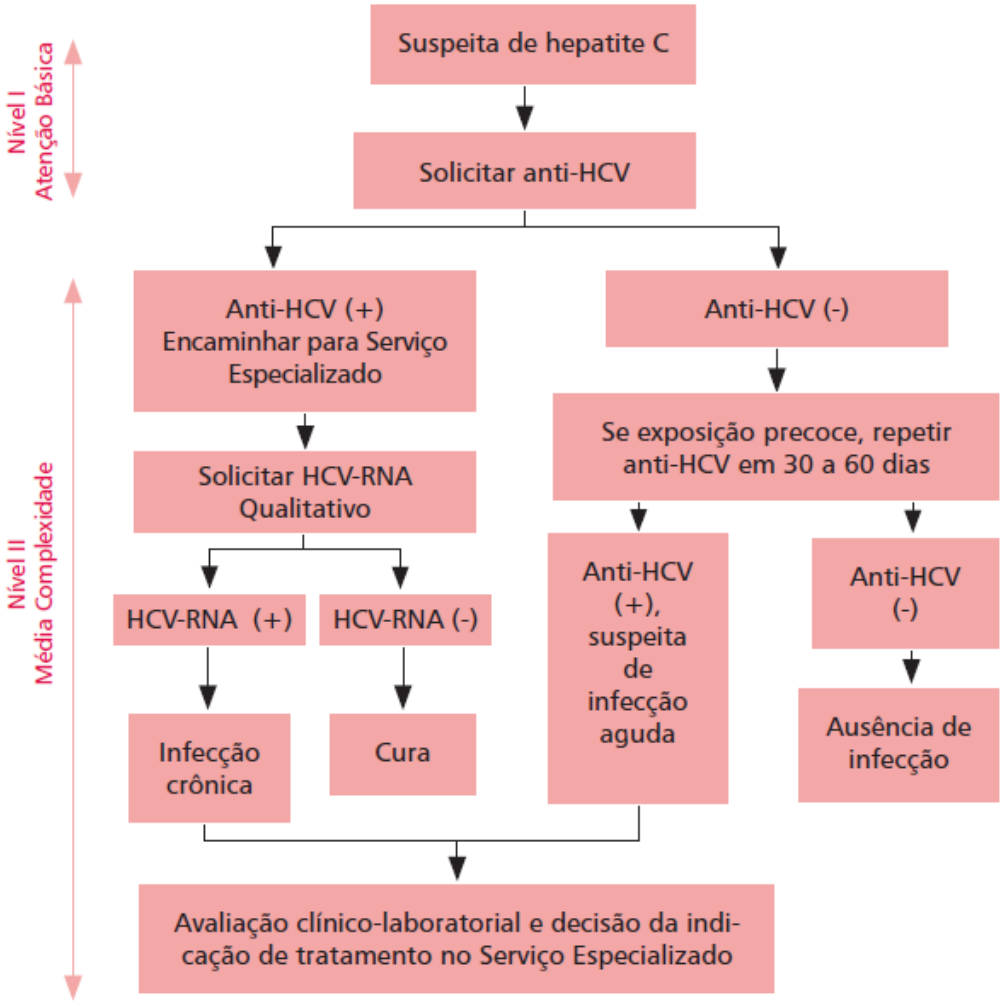
Fonte: RAMBO (2014)

Esse modelo assume que o HCV está associado a lipoproteínas de baixa densidade (LDL). Após a internalização o vírus perde o seu revestimento, expondo seu nucleocapisídeo e seu material genônico (RNA), na sequencia ocorre o mecanismo de transdução genômica, seguido pela replicação feita no retículo endoplamático. A fita de RNA recém-sintetizada é então empacotada pelo aparelho de Golgi em uma nova partícula lipoviral infecciosa, que então é liberada da célula. Os elementos de reconhecimento do microRNA (MRE 1,2) permitem a interação com o microRNA-122 do hospedeiro, facilitando a replicação viral (RAGHWANI et al., 2016).

2.3 MÉTODOS DE DETECÇÃO DA HEPATITE C NA ROTINA CLÍNICA

Os principais métodos de diagnóstico da Hepatite C tem como base a detecção direta do Anti-HCV no soro humano através de imunoensaios. A presença de anticorpos anti-HCV nem sempre significa a existência de infecção atual, pois pode representar resultado falso positivo ou indicar contato prévio com resolução (cura) da hepatite aguda, correspondendo a uma cicatriz imunológica (VANHOMMERIG et al., 2015).Assim, confirmada a presença dos anticorpos, é necessário a realização de ensaios moleculares para a detecção do RNA viral e diferenciação da fase da doenção (aguda ou crônica), os chamados testes de ácidos nucleicos (NAT, do inglês "Nucleic Acid Testing") (VILLAR et al., 2015). Na **Figura 5** é possível observar um fluxograma representando as etapas do diagnóstico do HCV.

Figura 5 - Fluxograma demonstrando as etapas do diagnóstico da Hepatite C.



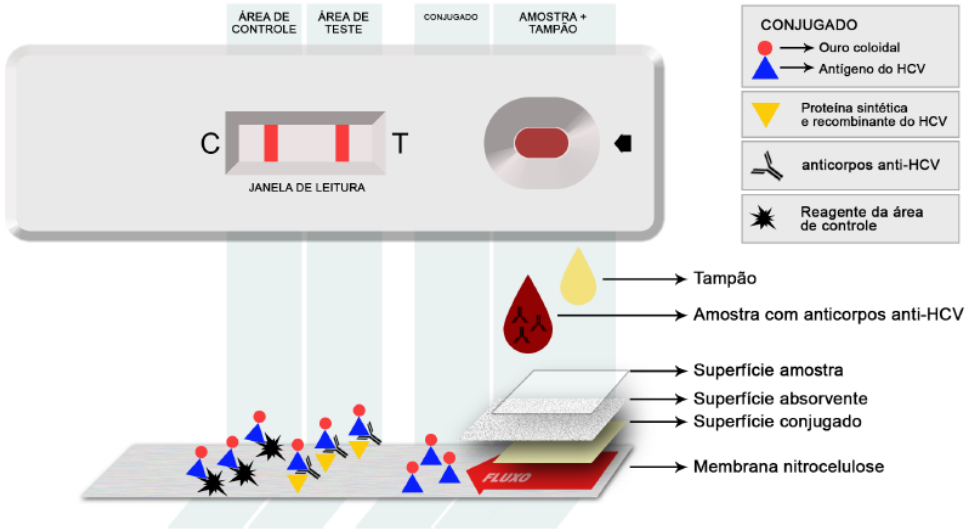
Fonte: BRASIL, 2017.

A disponibilidade de testes diagnósticos data de 1989, quando foi decodificado o genoma do HCV. A produção de antígenos e peptídeos sintéticos possibilitou o desenvolvimento de testes para detecção de anticorpos contra o HCV (anti-HCV), tais como os imunoensaios enzimáticos (ELISA, do inglês "*Enzyme-Linked Immunosorbent Assay")* e o ensaio imunoblot Recombinante (RIBA, do inglês "*recombinant immunoblot assay"*). Em sua primeira geração, o ELISA baseava-se na detecção apenas da proteína não-estrutural NS4 do HCV. O ELISA de segunda geração tinham como objetivo a detecção das proteínas virais NS3 e NS4. Atualmente, em sua terceira geração é possível detectar as proteínas NS3, NS4 e NS5, conferindo maior especificidade diagnóstica e ampliando a janela diagnóstica de 16 para 5 semanas da infecção (VILLAR et al., 2015, Aronoff-Spencer et al., 2016). Este método, em condições ideais, pode obter uma sensibilidade de aproximadamente 97%, porém serve apenas para indicar exposição prévia ao vírus, tendo como grande desvantagem os resultados falsos positivos, devido à existência de ligações inespecíficas entre as imunoglobulinas presentes no soro ou plasma e contaminantes das preparações antigênicas dos kits ou regiões não específicas dos antígenos recombinantes.

A fim de complementar o diagnóstico sorológico e avaliar possíveis resultados falso-positivos do teste ELISA, pode ser utilizada modificações da técnica de Western Blot, como o RIBA também para a detecção de anticorpos. Neste caso, a especificidade é alta, porém a sensibilidade é mais baixa que no teste ELISA. Além disso, os testes automatizados por imunoensaios por eletroquimioluminescência (ECLIA, do inglês "*Electrochemiluminescence Immunossay")* vêm substituindo atualmente o ELISA por sua praticidade, precisão e especificidade.

Testes diagnósticos rápido baseados no princípio dos imunoensaios de tira-lateral têm sido empregados para diagnóstico e triagem da hepatite C em unidades básicas de saúde. Esse sistema utiliza a técnica de imunocromatografia para detecção das proteínas do core e a estrutural 2 (E2) do capsídeo do HCV baseando-se no princípio da imunocaptura, onde dois anticorpos ligam epítopos distintos: anticorpo de detecção que liga complexo antígeno-anticorpo conjugado ao gerador de sinal (comumente utilizados são as partículas de látex ou ouro coloidal) e anticorpo de captura, imobilizado na superfície sólida para controle do teste **(Figura 6)**. Este sistema é bastante simples para leitura, entretanto o sinal colorimétrico gerado na imunocromatografia apenas estima qualitativa ou semi-quantitativamente os níveis sorológicos do anti-HCV. Adicionalmente, os testes de imunocromatográficos não podem ser considerados para um diagnóstico definitivo, uma vez que o resultado pode estar sujeito a um período de janela imunológica (CHEVALIEZ et al., 2016, KOSACK & NICK, 2016).

Figura 6 - Princípio metodológico do teste rápido de tira-lateral para diagnóstico do HCV.



Fonte: Disponível em https://telelab.aids.gov. br/moodle/pluginfile.php/22183/mod_resource/content/2/Hepatites%20-%20Manual%20Aula%204.pdf. Acessado em 30.05.2018

Os ensaios moleculares são realizados para confirmar a presença do agente viral e determinar a o estágio da infecção. Para tal diagnóstico são realizados testes baseados na reação em cadeia da polimerase (PCR, do inglês "*Polymerase Chain Reaction*") na qual é feita a amplificação de uma sequência específica de RNA viral (ROCKSTROH et al., 2017). Associado aos testes moleculares outros dados bioquímicos podem ser considerados para diagnóstico da hepatite C na fase crônica, tais como a dosagem da alanina-aminotransferases (ALT/TGP), os níveis de aspartato aminotransferases (AST/TGO) e de plaquetas, sendo mencionados como os melhores fatores preditivos de fibrose hepática. Outros exames de relevância para avaliação do órgão na fase crônica são as ultrassonografias e endoscopias digestiva alta (EDA) (LAMEIRA et al., 2013).

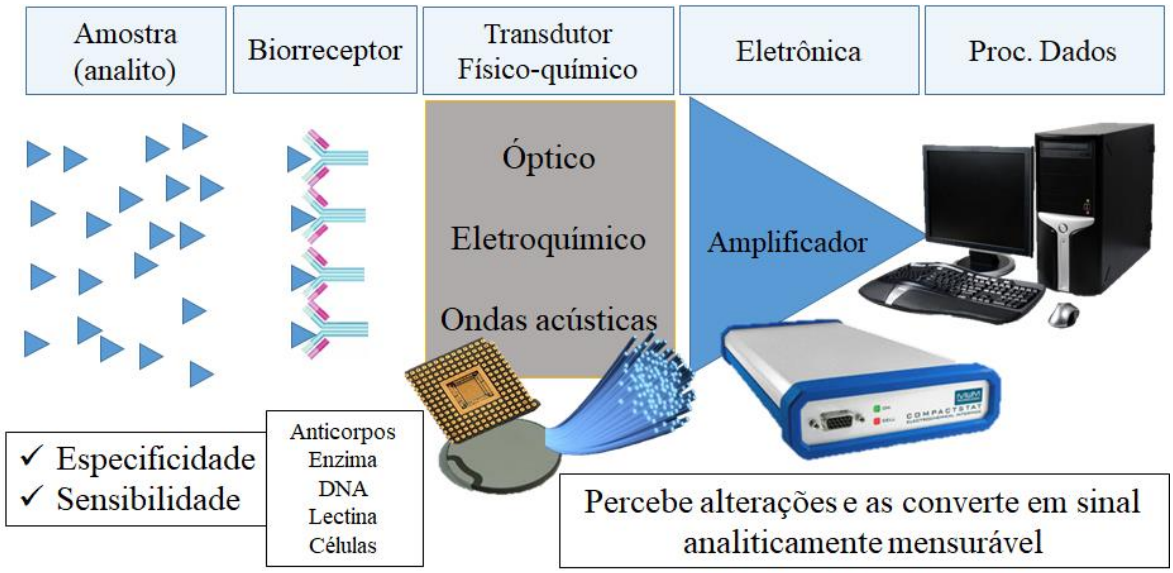
Diante do exposto, o desenvolvimento de testes mais rápidos e práticos, que dispensem a necessidade de processamento prévio da amostra (amplificação, extração, etc) e uso de profissional qualificado, é uma emergência no diagnóstico da hepatite C.

2.4 BIOSSENSORES

De acordo com a União Internacional de Química Pura e Aplicada (IUPAC, do inglês *"International Union of Pure and Applied Chemistry"*) biossensor pode ser definido como dispositivo analítico que utiliza um elemento de reconhecimento biológico, como biorreceptor específico para um analito de interesse, associado a um elemento transdutor, responsável por

transforma ou traduzir o sinal resultante da interação do analito em uma sinal elétrico quantificável nas mais variadas aplicações (LEE et al. 2011). A **Figura 7** apresenta um diagrama esquemático dos principais elementos que compõe um biossensor.

Figura 7- Diagrama esquemático de um biossensor.



Fonte: A AUTORA

Quanto ao elemento de bioreconhecimento podemos classificar os biossensores em: catalítico e de afinidade. Os biossensores catalíticos utilizam enzimas eletroativas como elemento de reconhecimento, mensurando produtos resultantes da reação enzimática na presença do seu substrato específico (RABA et al., 2013). Os biossensores de afinidade baseiam-se na formação de complexos entre o substrato e o receptor, tais como as interações antígeno-anticorpo (imunossensores), lectina-carboidrato e a hibridização entre sequências de ácidos nucleicos de fita simples (genossensores) (BERTÓK et al., 2013; PERUMAL & HASHIM, 2014).

Os biossensores podem ser classificados com base em dois parâmetros: no mecanismo de transdução e no elemento de bioreconhecimento. Com base nos mecanismos de transdução podemos classificar os biossensores como:

i) *óptico*: baseiam-se nas variações da resposta aos estímulos de luz como consequência da interação do analito de interesse com o elemento biológico. De acordo com o mecanismo de detecção, a quantificação do analito neste tipo de

transdução é realizada através de medidas do índice de refração, quantidade de luz absorvida, propriedades fluorescentes e fosforescentes (GUO, 2012);

ii) *piezelétricos:* detectam variações das frequências de ondas acústicas geradas por cristais piezelétricos em resposta à interação biomolécula-analito. Neste tipo de transdução, o cristal piezelétrico é submetido a um campo elétrico alternado que modifica o estado vibracional de oscilação harmônica do cristal, gerando uma onda acústica mensurável, comportando-se como uma microbalança para detecção da interação biomolécula-analito (MARRAZZA, 2014);

iii) *calorimétricos*: também chamados de termistor, detectam substratos baseados no calor envolvido nas reações bioquímicas do analito com uma substância biológica ativa como uma enzima (MEHROTRA, 2016);

iv) *eletroquímicos:* têm se destacado entre os tipos de transdutores mais empregados em biossensores. O princípio deste sistema de transdução baseia-se na mensuração de variações de uma propriedade elétrica resultante da interação analito-bioreceptor, podendo se classificado em: amperométrico, potenciométrico, condutimétrico e impedimétrico (BARSAN et al., 2015).

Nos últimos anos os transdutores eletroquímicos vêm ganhando destaque devido diversas vantagens, tais como facilidade de miniaturização, menor custo e compatibilidade com as tecnologias de produção em larga escala de sensores. Este tipo transdução desponta hoje como uma das principais tecnologias para o uso de testes de pronto-atendimento (POCT, do inglês *"Point-of-care testing"*), como exemplo prático destaca-se os *biossensores* de glicose vastamente utilizados na monitorização da diabetes (POHANKA et al., 2007; JIANG et al., 2017).

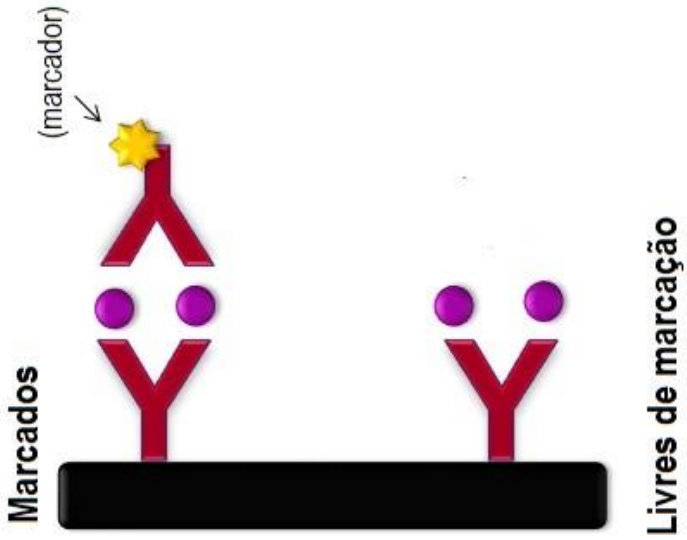
2.4.1 Imunossensores

Os biossensores baseados na interação antígeno-anticorpo como elementos de reconhecimento são chamados de imunossensores, que essencialmente combinam a sensibilidade e especificidade dos imunoensaios com a transdução de um sinal elétrico (LIU, 2015). O imunossensor, portanto, detecta a reação antígeno-anticorpo, sendo que o antígeno ou o anticorpo é imobilizado na superfície do transdutor. Na interação antígeno-anticorpo, um complexo é formado envolvendo ligações não covalentes como Van der Waals, ligação

eletrostática, pontes de hidrogênio e ligações hidrofóbicas. Estas ligações relativamente fracas ocorrem a curta distância, de modo que só as moléculas que contém o determinante antigênico interagem com o sítio ligante específico do respectivo anticorpo (ABBAS, 2012).

O monitoramento da interação antígeno-anticorpo nos transdutores eletroquímicos, em geral, utiliza espécies eletroativas como marcadores indiretos da detecção do analito, sendo denominados como imunossensores marcados. Tais espécies eletroativas participam indiretamente da reação conferindo maior seletividade analítica. Entretanto os sistemas marcados apresentam sensibilidade limitada para detecção de baixas concentrações e maior tempo de resposta, devido às diversas etapas de processamento. Assim, os imunossensores eletroquímicos surgem como tendência na área para o desenvolvimento de ensaios mais simples e rápidos. Os imunossensores livres de marcação independem do uso de conjugados aos anticorpos/antígenos, monitorando a interação através de mudanças de corrente, resistência ou capacitância diretamente na superfície da plataforma sensora (**Figura 8**) (BARSAN, 2015).

Figura 8 - Representação esquemática de um immunossensor



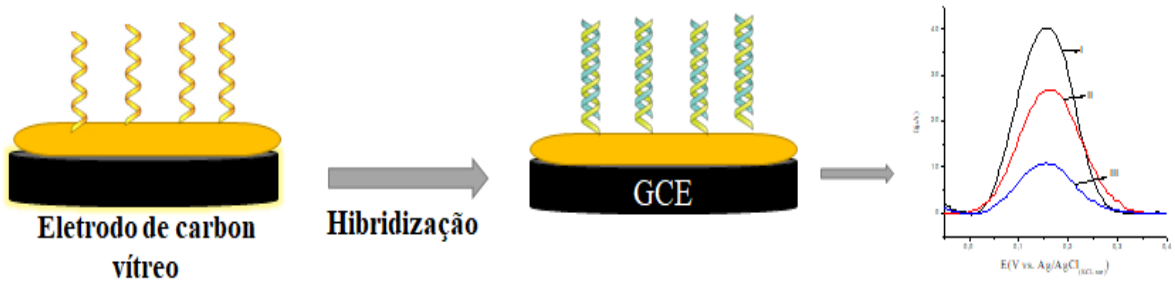
Fonte: A AUTORA

2.4.2 Genossensores

O desenvolvimento de um método de detecção de DNA sensível, específico e econômico está em alta demanda para o diagnóstico de doenças genéticas, como também na detecção de patógenos (vírus, bactérias, fungos, etc). Genossensor é definido como

dispositivo bioanalítico que utiliza como elemento de bioreconhecimento moléculas ou pequenos fragmentos de DNA ou RNA (**Figura 9**). Nos genossensores eletroquímicos a interação entre bases nitrogenadas específica são convertidas em sinais elétricos mensuráveis (potenciométricos, amperométricos, etc.), detectados de forma direta (*label-free*) ou indireta, através de marcadores eletroativos conjugados, tais como enzimas oxidoredutases, nanopartículas metálicas, fluoróforos, etc. Estes sensores são atrativos para as investigações e diagnósticos genéticos, bem como detecção de espécies patogênicas, analitos de interesse clínico, espécies carcinogênicas, fármacos e seqüências de bases de DNA (humano, vírus, bactéria, etc.) (SINGHAL et al., 2017)

Figura 9 - Representação do princípio de funcionamento de um genossensor.



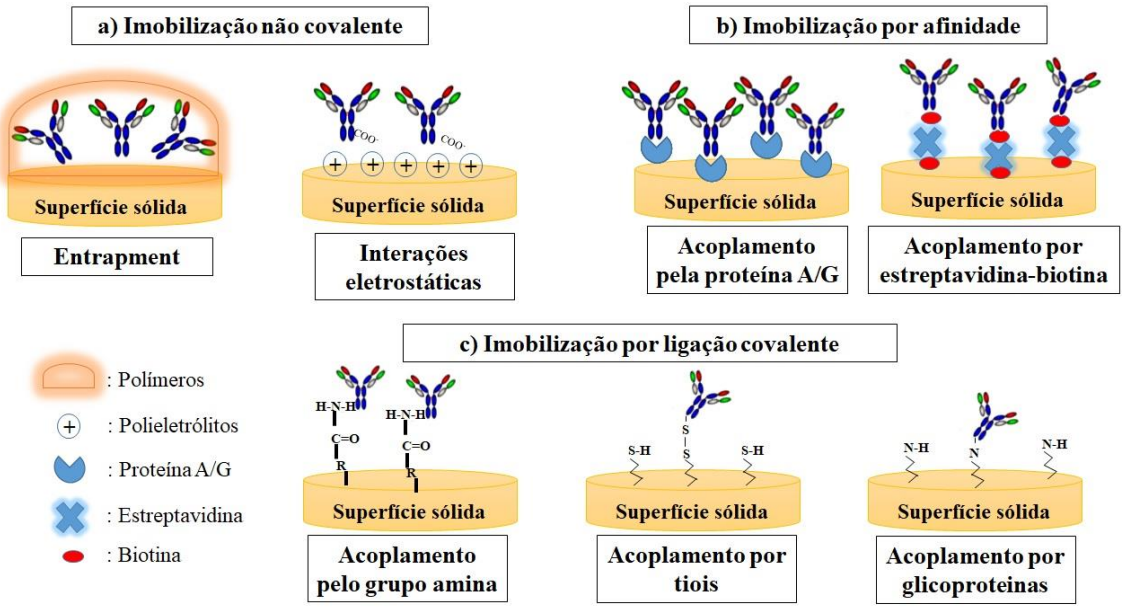
Fonte: A AUTORA

A transdução eletroquímica é um dos métodos analíticos amplamente utilizados em genossensores, pois fornecem uma plataforma de simples síntese, precisa e de baixo custo. Outra vantagem é a disponibilidade de uma ampla variedade de substratos de eletrodos e várias estratégias de modificação do eletrodo que possibilitam imobilização eficiente das sequências de DNA (CAMPOS-FERREIRA et al., 2016). A construção de um genossensor eletroquímico envolve, no geral, os seguintes passos: escolha do transdutor, imobilização do DNA e monitoramento eletroquímico do processo de hibridização (transdução do sinal). Cada passo deve ser otimizado, objetivando o melhor desempenho do genossensor. Para se alcançar uma boa sensibilidade e seletividade é requerido um processo de hibridização eficiente, com o mínimo de adsorção não específica sobre a superfície do eletrodo (RASHEED & SANDHYARANI, 2017).

2.5 MÉTODOS DE IMOBILIZAÇÃO EM IMUNOSSENSORES

A eficiência da imobilização de biomoléculas em imunossensores depende de muitos fatores, entre eles a superfície de contato e a presença de grupos funcionais específicos, sendo esses os grandes responsáveis pela retenção das biomoléculas nas plataformas sensoras (BARSAN et al.,2015). A etapa de imobilização afeta diretamente o limite de detecção do analito de interesse, a sensibilidade e o desempenho geral do imunossensor. Adicionalmente, a orientação adequada dos anticorpos sobre a surperfíce sensores e a modificação estrutural mínima enquanto imobilizado contribuem diretamente para o melhoria do desempenho do imunossensor . As principais abordagens descritas na literatura utilizam métodos de imobilização de anticorpos por: *i*) por interações não-covalente, baseado principalmente em interações eletrostáticas, tais como ligações iônicas, interações hidrofóbicas e forças de van der Waals entre o anticorpo e a superfície sensora **(Figura 10a)** *ii) reações de afinidade*, empregando proteínas intermediárias, tais como proteína A/G e avidina-abiotina **(Figura 10b)** ; e *iii) ligações covalente*, envolvendo a modificação de superfície da superície sensora com grupos reativos tais como grupos hidroxi, tiol, carboxi ou amino e subsequente imobilização do imunoreagente (**Figura 10c**) (SASSOLAS et al., 2012; LIU & YU, 2016).

Figura 10 - Representação esquemática de vários métodos de imobilização.



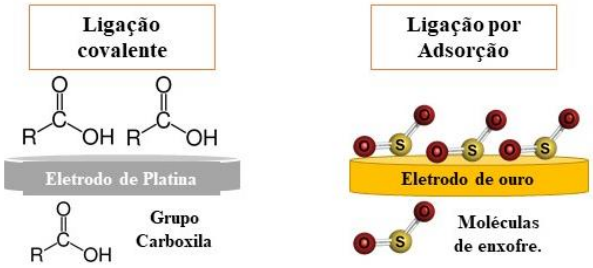
Fonte: A AUTORA

2.6 ELETRODOS QUIMICAMENTE MODIFICADOS

A modificação da superfície do eletrodo é considerada uma etapa essencial na construção de um biossensor eletroquímico, pois a partir dela é possível definir a conformação de molécula no processo de imobilização, aumentar a área eletroativa e melhorar a eficiência da cinética de transferência de elétrons. Assim, a otimização da estruturação da superfície sensora pode conferir ao biossensor características analíticas importantes, tais como, estabilidade, reprodutibilidade, sensibilidade e limites de detecção desejáveis (KANG et al, 2008; DUNG et al, 2012).

Os métodos de modificações eletródicas mais empregados são: *i) adsorção*, onde é possível depositar ou imergir substâncias de interesse na superfície do eletrodo empregando ligações fracas, tais como ligações iônicas, interações eletrostáticas, hidrofóbicas e forças de Van der Waals e *ii) ligações covalentes*, formada pela interação de grupos funcionais dos agentes modificantes na plataforma sensora formando ligações químicas estáveis (**Figura 11**) (SASSOLAS et al., 2012).

Figura 11 - Representação esquemática dos principais métodos de modificação da superfície eletródica.



Fonte: A AUTORA

Dentre os agentes que podem ser usados para modificar a superfície de eletrodos O uso de filmes poliméricos vem ganhando destaque na construção de sensores eletroquímicos.

Polímeros são definidos como material orgânico ou inorgânico de alto peso molecular, composto por um conjunto de cadeias poliméricas, sendo que cada uma dessas se trata de uma macromolécula que possui uma estrutura onde há repetição de unidades chamadas “meros” (CANDIAN, 2007). Esses podem modificar facilmente a superfície dos eletrodos, incorporando grupos funcionais que podem promover elevada afinidade e estabilidade do biocomponente. Além disso, as estruturas químicas dos filmes poliméricos são geralmente

passíveis de modificação e modelagem química, permitindo a alteração de suas propriedades elétricas, mecânicas e químicas (ZEN et al., 2003; MEDANY et al, 2012; GOMES-FILHO et al, 2013).

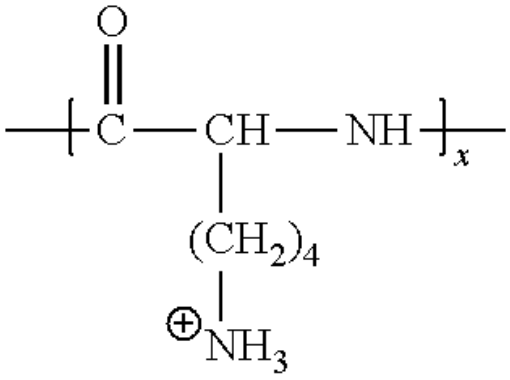
De acordo com a condução de corrente elétrica, os polímeros podem ser classificados em condutores e não condutores (SHRIVASTAVA et al., 2016). Nesse trabalho optou-se pela utilização da Poli-L-Lisina (PLL), poliaminoácido catiônico, e do Polipirrol (PPy, do inglês *"Polypyrrole"*), polímero orgânico condutor, para aplicação em diferentes abordagens de propostas de biossensores para detecção da Hepatite C.

2.7 POLI-L-LISINA

A PLL (**Figura 12**) é um polímero catiônico linear rico em grupamentos aminos, que permitem sua conjungação tanto com nanomateriais funcionalizados com grupos carbóxilos (-COOH) ou epóxis, (-O-), como com biomoléculas. A PLL é obtida pela condensação de várias unidades de seu monômero L-lisina, um aminoácido dotado de uma cadeia de hidrocarbonetos com cinco grupamentos $-CH_2$ e um grupo amino ($–NH_2$) na posição ε. De acordo com sua quiralidade, a lisina pode ser encontrada de duas formas poliméricas: a Poli-D-Lisina e a Poli-L-Lisina. A PLL pode ser obtida sobre diversos pesos moleculares resultantes do número de unidades matriciais utilizadas para sua confecção (MILLONE et al., 2016; SAHINER, 2017; THIRUMALRAJ et al., 2017).

Nos últimos anos a PLL vem sendo explorada na construção de sensores eletroquímicos, pois apresenta características interessantes para a modificação de superfícies eletródicas, tais como biocompatíbilidade, biodegrabilidade, baixa toxicidade e antigenicidade, sendo facilmente solubilizado em água (MILLONE et al., 2016). Este polímero, quando utilizado no revestimento de superfícies eletródicas é capaz de promover interação com ânions, uma vez que é dotado de um grupamento amino-protonado em valores de pH abaixo de 11,0 (pKa= 10,7) e a possibilidade de complexação pela amina desprotonada em valores de pH superiores. (GUO et al., 2015)

Figura 12 - Estrutura química da PLL.



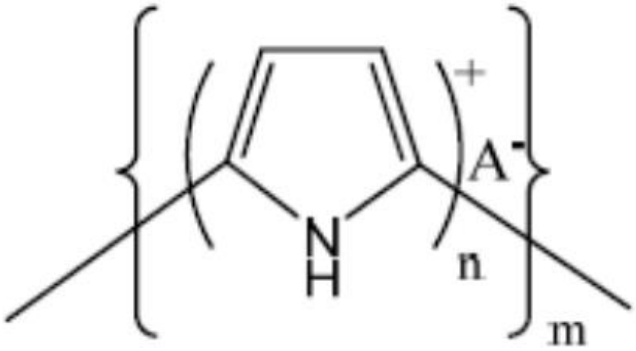
Fonte: Disponível em <http://www.google.com/patents/US8574384>. Acessado em: 18/10/2017.

As reações de formação do polímero ocorrem entre as unidades monoméricas da L-lisina, através da formação de ligações peptídicas. A estrutura final da PLL apresenta ligações peptídicas e grupos amino ativos (NH_3^+) abundantes nas cadeias laterais, em pH neutro (HE et al., 2015). O polímero formado sobre a superfície do eletrodo apresenta uma natureza fortemente catiônica que possibilita maior propagação de cargas no filme sendo favorável ao rápido movimento dos contra-íons e favorece, assim, a transferência eletrônica entre a superfície do eletrodo e as espécies carregadas em solução. (THIRUMALRAJ et al., 2017)

2.8 POLIPIRROL

Dentre os polímeros condutores existentes, o PPy (**Figura 13**) é um dos mais estudados, pois apresenta características intrínsecas interessantes, tais como excelentes propriedades física, elétricas, óticas e alta estabilidade química. Além disso, o PPy pode ser oxidado a relativamente potenciais baixos, quando comparado aos outros monômeros (tiofeno, anilina, etc) e em meios aquosos e orgânicos (AYDEMIR et al., 2016; AFZAL et al., 2017).

Figura 13 - Estrutura química do PPy.



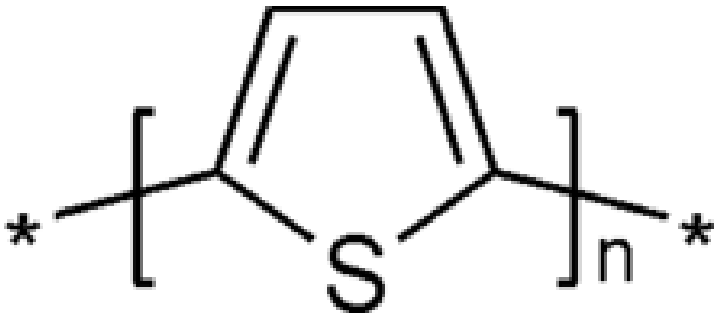
Fonte: ZHAN et al. (2017).

A síntese do PPy é obtida por duas vias principais, nas quais o monômero de Pirrol pode ser polimerizado quimicamente e eletroquimicamente. Na síntese química é possível obter o PPy na forma de pó e em grande quantidade, a um baixo custo. Entretanto, a polimerização eletroquímica é a mais empregada uma vez que através dela é possível obter um controle maior da espessura do filme, preservando ao máximo suas propriedades elétricas e possibilitando a formação de um polímero mais homogêneoestruturalmente diretamente sobre a superfície do eletrodo (HUANG et al., 2016). A obtenção do PPy via polimerização eletroquímica inicia-se através da oxidação dos compostos monoméricos em potenciais específicos, culminando com a formação de um radical catiônico extremamente reativo. Este radical reage com outro radical catiônico monomérico, através do acoplamento oxidativo, liberando dois prótons. A propagação da cadeia segue a seguinte sequência: oxidação, acoplamento e liberação de prótons, formando oligômeros e posteriormente polímero (AFZAL et al., 2017; MOOSAEI et al., 2017).

2.9 POLITIOFENO

Outra família de polímeros condutores são os politiofenos, eles são derivados da polimerizados do tiofeno, uma molécula heterocíclica de enxofre **(Figura 14).**

Figura 14 - Estrutura química do Politiofeno.



Fonte: ALVES et al., (2017).

Politiofenos e seus derivados, obtidos através de polimerização química ou eletroquímica a partir de seus respectivos monômeros, têm sido alvo de um amplo número de estudos devido às promissoras aplicações. Diferentes modificações químicas têm sido realizadas, nos últimos anos, com o intuito de melhorar as propriedades destes polímeros conjugados para satisfazer as diversas características necessárias para aplicações em dispositivos eletrônicos orgânicos. Estudos sobre a estrutura eletrônica do tiofeno, foram realizados para elucidar o papel que os grupos dopantes e exercem nas propriedades eletronicas do material polimérico, no qual o band gap pode ser alterado de 3 para 1 V com base no nível dos grupos dopantes empregado. Além disso, alterando a morfologia e, portanto, a geometria das cadeias de oligômeros, o band gap e as propriedades ópticas dos filmes baseados em politiofeno pode ser drasticamente ser alterado (ALVES et al., 2014).

2.9 NANOMATERIAIS DE CARBONO

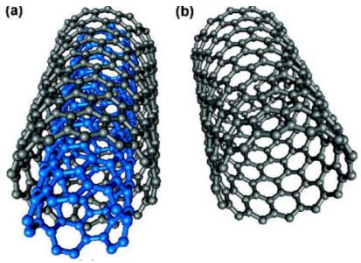
Os nanomateriais de carbono tem se destacado como um dos mais interessantes materiais aplicados em biossensores eletroquímicos nos últimos anos. Eles podem combinar propriedades como alta área superficial, biocompatibilidade, estabilidade química e eletroquímica e boa condutividade elétrica (ZHU et al., 2012). Nessa classe pode-se destacar os Nanotubos de Carbono (NTCs) e o Oxido de Grafeno, estes são extremamente atraentes na área bioanalítica para o desenvolvimento de plataformas sensoras e foram utilizados nesta tese em diferentes abordagens de filmes compósitos nanoestruturados.

2.9.1 Nanotubo de Carbono

Os NTCs, descobertos em 1991 por Sumio Iijima, são uma classe de nanomateriais que apresentam extraordinárias propriedades mecânicas, elétricas e térmicas. A estrutura dos NTCs pode ser descrita como um enrolado invólucro tubular de folha de grafite com os átomos de carbono covalentemente ligados aos seus vizinhos (SILVA et al. 2014). Quando aplicados em sensores eletroquímicos, os nanotubos podem promover uma rápida transferência de elétrons com maior eficiência, diminuindo o valor de sobrepotencial de trabalho para vários substratos eletroativos, aumentando a velocidade da reação de muitas espécies eletroativas e, em seguida, a diminuição do tempo de resposta do sensor, possibilitando assim, alcançar alta sensibilidade com baixos limites de detecção. Os NTCs têm atraído uma série de esforços de pesquisa básica, podendo ser aplicados para fins biomédicos, como a entrega de drogas, nanoinjetores, fototerapia e produção de imagens artificiais (KRUSS et al., 2013; FREITAS et al. 2014; GOMES-FILHO et al. 2013).

Do ponto de vista estrutural existem dois tipos de NTCs (**Figura 15**), os de parede simples (SWCNT do inglês *single-walled nanotubes*), compreendendo uma única folha de GF enrolada sobre si, e os de múltiplas paredes (MWCNT do inglês *multi-walled nanotubes*), podendo ser entendido como, um conjunto de três ou mais NTCs concêntricos enrolados sobre si (FREITAS et al. 2014; GOMES-FILHO et al. 2013). Estes últimos apresentam vantagens, devido ao fato de frequentemente possuírem baixo custo e síntese mais simplificada, o que os torna mais acessíveis (WU, et al., 2007).

Figura 15 - Representação estrutural dos NTC, (a) múltiplas paredes; (b) parede simples.



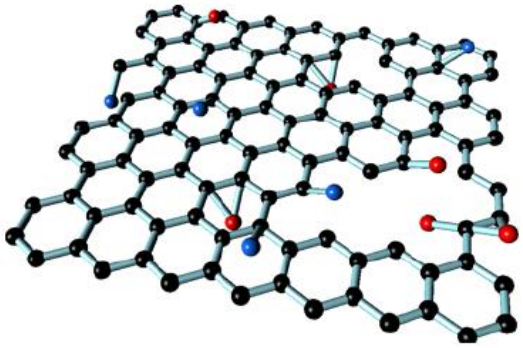
Fonte: WASIK et al. (2017).

A funcionalização da superfície dos NTCs, associando a eles grupos funcionais proteína-reativos, tem sido um ponto fundamental para a conjugação de biomoléculas como elemento de reconhecimento (DIAS et al. 2013). Para fazer a funcionalização dos nanotubos vários compostos podem ser utilizados, tais como grupos químicos ou metais de transição (FILHO, et al., 2007), existe ainda a possibilidade de modificação através da interação de polímeros com suas paredes (WANG et al., 2016). Com isso, área superficial e os grupos reativos possibilitam aos NTCs podem ser utilizados para a imobilização de moléculas pequenas ou moléculas biologicamente ativas em superfícies sensoras.

2.9.2 Óxido de Grafeno

Na última década o grafeno vem despontando como um importante nanomaterial para o uso na nanotecnologia. De acordo com suas características físicas e químicas,pode-se encontrar várias formas de grafeno, dentre elas destaca-se o Óxido de Grafeno (GO, do inglês "*Graphene Oxide"*), devido a riqueza de grupos funcioanais bastante atrativos para a montagem de superfícies sensoras (SADEGH, 2017).O GO possui duas dimensões (**Figura 16**), sendo composto por uma rede hexagonal de ligações sp^2 entre átomos de carbono (C-C) e também por ligações sp^3 com átomos de oxigênio (C-O) formando grupamentos carboxilas (-COOH), hidroxilas (-OH) ou epóxi (-O-) (GUEX et al., 2017).Dentre as suas características mais notáveis do GO podemos citar, uma grande área de superfície com cerca de 2600 m^2/g, propriedades superficiais únicas, boa dispersão em água e propriedades ópticas (KIEW et al., 2016). A utilização do GO vai desde ação antibactericida (PALMIERI et al.,2017), uso como *drug delivery*, até a formação de plataformas sensoras nanoestruturadas (DAI et al., 2016).

Figura 16: Formas estrutural do GO.

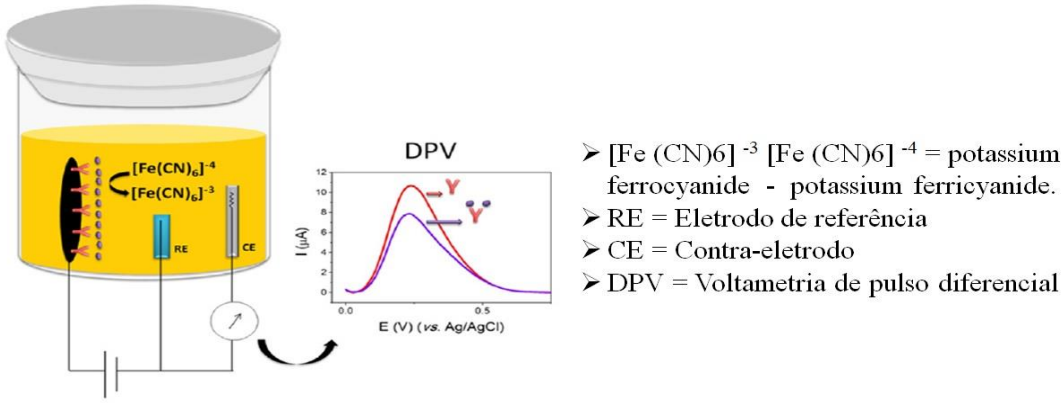


Fonte: GUEX et al. (2017).

2.10 TÉCNICAS DE CARACTERIZAÇÃO ELETROQUÍMICA

Técnicas de caracterização e análise eletroquímicas apresentam inúmeras vantagens quando aplicadas na construção de plataformas sensoras, podendo citar-se a economia adquiridas pela utilização de pequenas quantidades de reagentes nas análises, além da possibilidade manipulação e combinação de inúmeras variáveis a partir de técnicas como: voltametria cíclica (VC), voltametria de onda quadrada (VOQ), voltametria de pulso diferencial (VPD), impedância e cronoamperometria. Tais técnicas fazem uso de propriedades elétricas mensuráveis (corrente, potencial e carga) de um analito quando este é submetido a uma diferença de potencial entre eletrodos em uma célula eletroquímica **(Figura 17)**. Essas medidas podem então ser relacionadas com algum parâmetro químico intrínseco do analito (SHARMA et al., 2012).

Figura 17 - Representação esquemática de uma célula eletroquímica trieletródica.



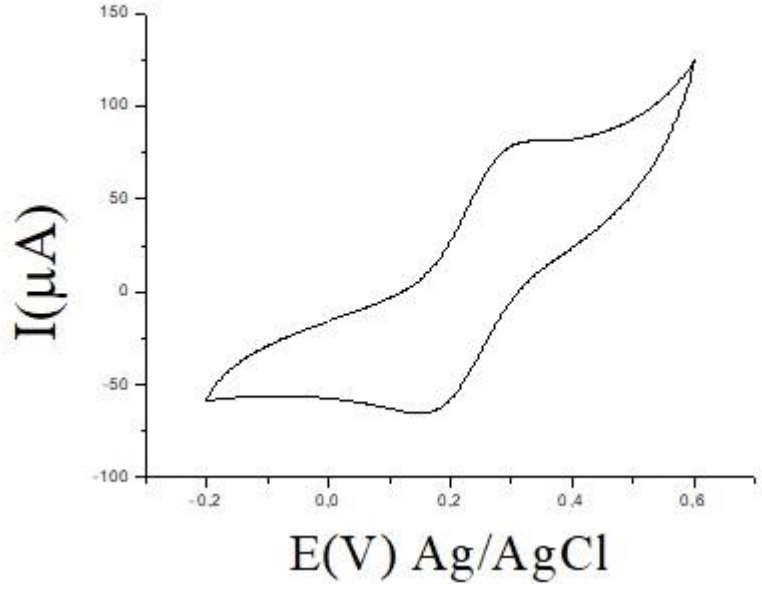
Fonte: Adaptado de SILVA et al., 2013.

2.10.1 Voltametria Cíclica

A voltametria cíclica (CV) é uma técnica eletroanalítica versátil para o estudo e monitoramento de espécie de interesse. A VC acompanha o comportamento redox de espécies eletroquímicas dentro de uma ampla faixa de potencial. A corrente no eletrodo de trabalho é monitorada em um potencial de excitação triangular aplicado ao eletrodo. O voltamograma resultante pode ser analisado quanto às informações sobre a reação redox. Voltamogramas cíclicos são os equivalentes eletroquímicos aos espectros em espectroscopia óptica (LOWINSOHN & BERTOTTI, 2006).

Na VC o potencial aplicado ao eletrodo é variado numa velocidade conhecida, e ao atingir o potencial final desejado, a varredura é revertida ao valor inicial, na mesma velocidade (**Figura 18**). Obtém-se como resposta a essa perturbação, por exemplo, um par de picos catódicos e anódicos, cujos parâmetros eletroquímicos mais importantes, são os potenciais de pico catódico e anódico (Epc e Epa), as correntes de pico catódico e anódico (Ipc e Ipa), e os potenciais de meia onda (E1/2), essenciais para caracterizar o processo eletródico ocorrido (PACHECO et al., 2013).

Figura 18 - Representação esquemática de uma voltametria cíclica.



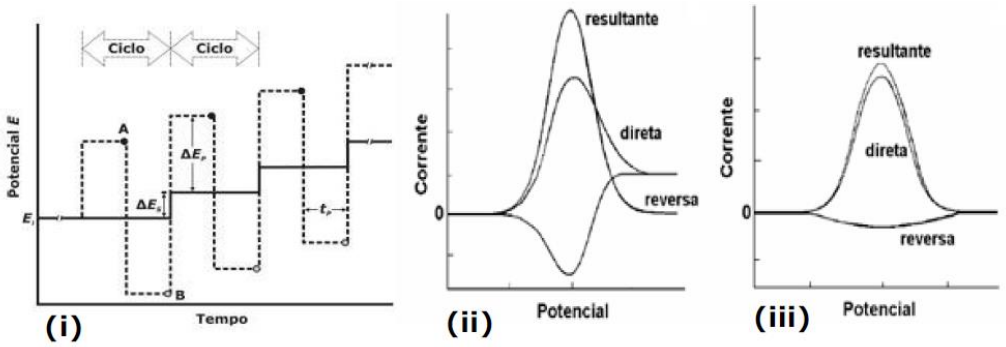
Fonte: A AUTORA

2.10.2 Voltametria de Onda quadrada

A VOQ é uma técnica onde a variação de potencial é realizada na forma de uma escada, na qual pulsos de potencial (ΔEs) de igual amplitude são sobrepostos a uma escada de potenciais de altura constante (ΔEp) e duração 2tp (período). A corrente elétrica é medida ao final dos pulsos direto (A-catódico) e reverso (B-anódico), originando um pico simétrico com posição, largura e altura característicos do sistema avaliado (ΔI), o qual é um sinal obtido diferencialmente, e apresenta excelente sensibilidade e alta rejeição a correntes capacitivas

(WANG et al., 2019). Na **Figura 19**, pode-se observar o sinal de excitação e o voltamograma típico para análises VOQ.

Figura 19 - Demonstração esquemática do processo de registro de voltamogramas de onda quadrada.



Fonte: SILVA, 2016

2.11 BIOSSENSORES PARA A DETECÇÃO DA HEPATITE C

A utilização de biossensores como ferramenta para o diagnóstico na área de clínica é uma das linhas de pesquisa em tecnologias mais interessantes. Estima-se que a indústria de Biosensor cresça em uma taxa composta anual de crescimento (CAGR, do inglês "*Compound Annual Growth Rate"*) de 9,6% de 2014 a 2020. Dentre as principais formas de desenvolvimento de biossensores os baseados nas ligações de afinidade, tais como entre antígeno e anticorpo e a hibridização entre as fitas de DNA, vem ganhando destaque no desenvolvimento de testes de pronto-atendimento, pois garantem resultados rápidos com grande sensibilidade. Existe, atualmente, uma intensa busca por dispositivos sensores capazes de identificar o vírus da Hepatite C em pacientes de maneira mais rápida, evitando, assim, maiores complicações para a saúde do paciente e também para que este não contamine outros por ignorar a doença da qual é portador (JOYCE et al, 2010; FOCACCIA, 2013).

Atualmente, propostas de imunossensores para detecção da HCV têm sido registrados na literatura envolvendo diferentes métodos de transdução. Dentre estes, estão reportados os imunossensores e genossensores (**Figura 20**).

Figura 20: Testes para a HCV encontrados.

REFERÊNCIA	PRINCÍPIO DE TRANSDUÇÃO	ELEMENTO DE RECONHECIMENTO	LIMITE DE DETECÇÃO
Singhal *et. al.*, 2016	Impedimétrico	Genosensor	90 copias/mL.
Hejaz et al., 2010	Eletroquímico	Genosensor	60 nM
Lu et al., 2015	Eletroquímico	Genosensor	2,3 pM
MORAES *et. al.*, 2013	Eletroquímico	Imunossensor	0,1 µg/mL
Aronoff-Spencer *et. al.*, 2018	Eletroquímico	Imunossensor	12,3 ng/µL

Fonte: A AUTORA.

3 RESULTADOS

5.1 ARTIGO 1 - Hepatitis C screening testing based on electrochemical nanoimmunosensor one-step eletrosyntetized

Artigo à ser submetido no periódico *Talanta*

Impact factor: 4.162

Hepatitis C screening testing based on electrochemical nanoimmunosensor one-step eletrosyntetized

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ABSTRACT

A novel label-free electrochemical immunosensor was developed for anti-HCV quantification, an important biomarker of the Hepatitis C virus infections. In order to amplify of the electrochemical signal for anti-HCV detection, a graphene oxide-polypyrrole (GO@PPy) nanocomposite film was assembled on glass carbon electrode. GO nanosheets were attached into the PPy matrix in a one-step of synthesis by using the cyclic voltammetric technique as procedure electropolymerization. Then, the high avidin-biotin affinity was used for directed immobilization of the HCV antigen on sensor surface. The label-free anti-HCV detection was obtained directly by square-wave voltammetry measurements in a ferri/ferrocyanide solution, as redox probe. The synergic effects of the GO nanosheets and PPy as nanocomposite film increase the electroactive area in 64.5%, demonstrating a good electrochemical stability (0.75%). Under optimal conditions, the immunosensor exhibited a linear range from 0.2 to 14 $ng.mL^{-1}$ with a low detection limit of 1.63 $ng.mL^{-1}$ for anti-HCV, as compared with traditional analytical method. The immunosensor presents a great potential for the ultra-sensitive detection of anti-HCV at levels of clinical importance, helping in the hepatitis C diagnostic and prevention of the HCV transmission through blood bags screening.

Keywords: polypyrrole; graphene oxide; label-free detection; hepatitis C; amperometric immunosensor.

1. Introduction

Hepatitis C virus (HCV) infection is an increasing public health problem, associated with progressive liver damage with high rates of morbidities and mortalities. According to the World Health Organization, approximately 3% of the world population are infected with hepatitis C virus (HCV) with 3–4 million infections annually and at least 150 million chronic carriers at risk of developing liver cirrhosis and hepatocellular carcinoma [1]. One of several strategies for prevention of HCV transmission is screening for anti-HCV serology among donated blood in the blood bank [2]. A positive anti-HCV test also can be used as differential diagnostic for active HCV infection, chronic phase diagnostic of the HCV infection and false positive results [3].

The detection of anti-HCV is routinely performed by using mainly the enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence assay (ECLIA) [4]. Although well established in the clinical routine, the conventional immunoassays require long processing in a laboratory unit, poor user-friendliness, technical complexity and time-consuming [5]. Recently, indirect lateral-flow tests have been developed as point-of-care testing for anti-HCV detection in blood or oral fluids, with results restricted to positive or negative reaction, without anti-HCV quantification, and limited specificity in clinical applications [6, 7]. Thus, the development of immunosensing tool compatible with the technologies of point-of-care testing's has emerged as an alternative analytical tool for rapid anti-HCV detection.

The electrochemical transducers are frequently designed for development of the point-of-care testing in immunosensor, due to their portability, low cost, simplicity of instrumentation and ease of operation. However, the small current response produced by antigen-antibody recognition can be reduced the sensitivity, needing of an efficient signal amplification strategy for the immunosensor. In this perspective, carbon nanomaterials have been an important strategy for improvement of the electron-transfer kinetics in electrochemical detection. Among them, graphene oxide (GO) is the most commonly used carbon nanomaterials in electrochemical immunosensor, ascribed to their facile synthesis, high surface area and excellent biocompatibility. However, the GO has a moderate conductivity because of the disruption of its sp^2 bonding by functional groups [8, 9, 10]. An alternative to improve the GO electrical conduction is the inclusion of the nano-sheets in a conductive polymers matrix, resulting in a nanocomposite film [11, 12].

The use of GO together with conductive polymers as a single building block for supramolecular assembly to form various materials has been widely employed, especially since they enable the three-dimensional assembly of GO sheets, what possibillita synergistic properties like enhancement in electrical conductivity and electrochemical cyclability, to form thin-films [13, 14]. Polypyrrole, among the conducting polymers is specifically advantageous allowing the formation of thin, uniform and highly conductive films, It can be used in the manufacture of chemical sensors, biosensors and supercapacitors [15]. Composite films consisting of GO and polypyrrole (PPy), through the presence of π-bond on the pyrrole ring, which can be adsorbed on the GO surface by π-π, exhibited good electrochemical properties and cycling performance, which should be promisingly used for the fabrication of inexpensive, high-performance electrochemical supercapacitors [16].

As compared with traditional chemical synthesis, the electrosynthesis of nanocomposites is an attractive method due to its simple and fast nature in the preparation of the polymer nanocomposites, exploiting graphene-related materials [17]. Electrosynthesis of the conductive nanocomposites restricts the reactions on the electrode surfaces and can be controlled easily by changes in the applied potential or current density [18]. It provides an effective and convenient one-step approach to produce reproducible and stable thin-films with controlled morphologies and properties [19]. In this study, a conductive nanocomposite film based on PPy and GO was assembled on glassy carbon electrodes via a simple and controllable one-step electrosynthesis with proposal for anti-HCV detection in HCV infections.

2. Material and methods

2.1 Reagents

Py monomers (98%, v/v), GO in aqueous solution (0.2 µg mL^{-1}), Streptavidin (SA), glycine, potassium ferrocyanide ($K_4Fe(CN)_6$), potassium ferricyanide ($K_3Fe(CN)_6$), N-hydroxysuccinimide (NHS) and N-ethyl-N′-(3-dimethylami-nopropyl) carbodiimide (EDC) were obtained from Merck (St.Louis, USA).The biotynilated HCV antigen (HCVcAg) and monoclonal human anti-HCV were purchased from Abcam (Cambridge, UK).

The phosphate buffered saline (PBS) (0.01 mol L^{-1}, pH 7.4) was used in all dilutions of protein samples. The ultrapure water was obtained from a water purification system Milli-

Q (Billerica, USA) (18MΩ) and it was utilized to prepare all solutions. All chemicals used in the study were of analytical reagent grade purity.

2.2 Apparatus

The electrochemical experiments were carried out by using an Autolab PGSTAT12 potentiostat/galvanostat (Eco Chemie, The Netherlands) interfaced to PC computer system and controlled by Autolab software NOVA (2.1.2). It was used a three electrode system, comprising a glassy carbon electrode (GCE) (Ø = 3 mm) as the working electrode, a helical platinum wire as counter electrode and Ag/AgCl $_{(KCl\ saturated)}$ as the reference electrode. Electrochemical analyses were performed in an electrochemical cell (10mL) and conducted at room temperature (~ 24 °C).

The structural characterization was accomplished with Fourier Transform Infrared in the Attenuated Total Reflectance mode (ATR FT-IR) by using the Bruker IFS 66 model FT-IR spectrometer (Billerica, USA). Spectra were acquired at 4000-500 cm^{-1}. Scanning electron microscopy (SEM) assays were performed in the Scanning Electron Microscope JEOL - JSM 5600LV. A glassy carbon disc (~ 0.5 cm diameter) was adapted in a electrochemical cell for performed structural and morphological characterizations.

2.3 Preparation of the PPy@GO nanoelectrode

Prior to modification, the GCE was polished with alumina powder (1 and 0.5 mm) for 3 min until obtaining a mirror like surface. In order to remove any impurities, the cleaned electrode was carefully rinsed with ultrapure water. Then, the GCE was immersed in a mix solution of the Py monomers (0.3 $mmolL^{-1}$) and GO (0.2 mg mL^{-1}) and submitted to electropolymerization procedure. It was performed in a single one-step of synthesis by using the cyclic voltammetry technique in a potential window of -0.8 to 0.8 V, by 20 cycles at 20 mV s^{-1} scan rate.

2.4 HCV antigen immobilization

The functional groups derived of the PPy@GO nanocomposite were activated by incubation with EDC/NHS solution (0.02/0.05 mol L^{-1}) prepared in deionized water during 1 h at room temperature. Then, the SA (SA) (10 µg mL^{-1}) was covalently immobilized on the nanocomposite film in order to conjugate the biotinylated HCVcAg on the sensor surface. An aliquot (5 µL) of the HCV solution (100 µg mL^{-1}) was dropped on to the electrode surface and incubated for 1 h at room temperature. Non-specific bindings were blocked by incubating the GCE surface in a solution of glycine (0.05 mol L^{-1}), prepared in ultra-pure water, during 40min.

2.5. Analytical response

The label-free electrochemical immunoassay was based on the diffusion of $[Fe(CN)6]^{3/4-}$ redox probe through PPy@GO nanocomposite during the antigen-antibody recognition event. It was monitored by square wave voltammetry (SWV) measurements. These measures were recorded between 0.0 and 0.5 V at 10Hz with pulse amplitude of 10 mV and step potential of 2.5 mV. The detection of antibodies against HCV was standardized by using the perceptual decrease of current (ΔI %) in the SWV measurements of the HCV-modified electrode before (blank) and after incubations with different antibodies concentrations. All measurements were performed in triplicate.

3. Results and discussion

3.1 Characterization of the PPy@GO film on the GCE

As one of the most common electrochemical detection methods, cyclic voltammetry (CV) with $K_3Fe(CN)_6/K_4Fe(CN)_6$ (0.005 mol L^{-1}), could be not only used for the surface characterizations of the electrodes and detection, but also used as excellent electrode modification methods. In figure 1A it is possible to observe the electrochemical behavior of the different nanomaterials used in the construction of the film. When the GO (curve II) is deposited on the EGC surface, a decrease in the current peak is observed, compared to the clean electrode (curve I) by the presence of highly electrical resistant groups like carboxyl,

hydroxyl, or epoxy in GO sheets [20]. After the electropolymerization of Py monomer on the surface of the electrode (curve III), an increase in the current peak was observed in relation to the clean electrode (curve I), explained by the conductive nature of the polymer. The conductivity is derived from the high number of electrons in the formation of longer chain of the PPy in the polymerization process [21]. It was observed the electrochemical profile of the film formed by the electropolymerization of the PPy-GO, we observed an increase in the current peak in relation to the clean electrode (curve I) and the only nanomaterial (curves II and III), suggesting that the formation of the nanocomposite occurred and there was an improvement in its electrochemical characteristics. This increase in current peak can be explained by the capacitance originates from the double-layer capacitance of graphene and the pseudocapacitance of PPy. Due to the large aspect ratio and surface area of the GO sheets, they may serve as effective percolative conducting bridges and thereby increase the conductivity of PPy/GO composite at low GO content. In this process, the relatively large anionic GO serves as a weak electrolyte and is entrapped in the PPy nanocomposites during the electropolymerization of pyrrole, and also acts as an effective charge-balancing dopant within the PPy film. Through the technique of cyclic voltammetry it is also possible to determine the surface area that contributes to the current gain. The calculation of this electroactive area is carried out through the equation of Randles-Sevcik (equation 1) which describes the effect of scan rate on the peak current ip. In figure B it is possible to observe the bar graph showing the relation between the area of the cyclic voltammograms obtained through the interaction of the different nanomaterials that form the film and the CGE. Compared with the area obtained by the GO and PPy separately the film formed by the nanocomposite of PPy @ GO obtained an increase of 88.23% and 35.29% respectively, This could be interpreted by the fact that the film formed on the surface of the GCE improves the diffusion of $K_3Fe(CN)_6/K_4Fe(CN)_6$ (0.005 mol L^{-1}).

$$Ip,a = 2,69x105 \; . \; n3/2. \; A. \; Do1/2.[Ox].v1/2 \qquad (1)$$

where: Ip, a = anode current / A; n = number of electrons involved = 1; A = electroactive area / cm2; Do = diffusion coefficient of [K3Fe (CN) 6] = 7.70 x 10-6 cm2 s-1; [Ox] = [K3Fe (CN) 6] cell = 5.00 mmol L-1 and ν = sweep speed / V s-1.

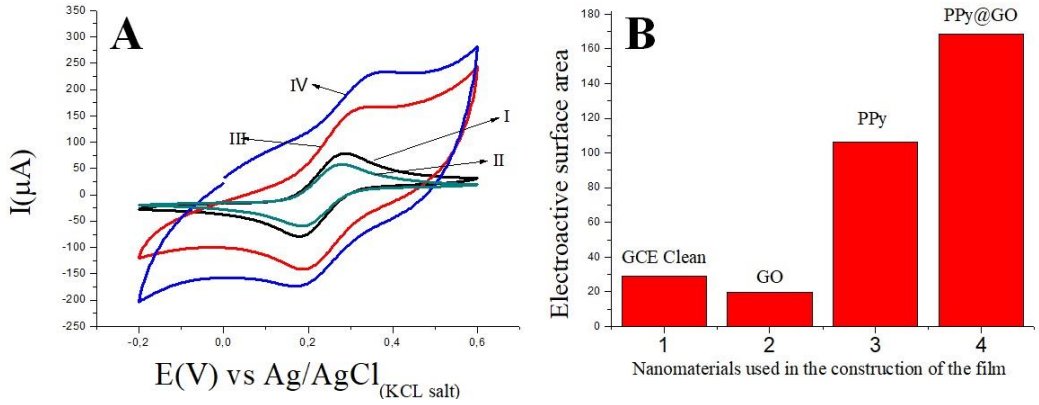

Fig 1.a) Cyclic voltammetry of the (I) GCE after cleaning; (II) GO/GCE; (III) Ppy/GCE; (IV) Ppy-GO/GCE; b) bar graph demonstrating the electroactive areas of the different materials that make up the film, (1) GCE after cleaning; (2) GO/GCE; (3) Ppy/GCE; (4) Ppy-GO/GCE.Measurements performed in $K_3Fe(CN)_6/K_4Fe(CN)_6$ (0.005 mol L^{-1}) prepared in KCl solution (0.1 mol L^{-1}).

The SEM micrographs were employed to evaluate the morphology of electrode surface. In Fig. 2A and 2D the micrograph of the Ppy revealed a uniform granular structure. In Fig. 2B and 2E, the micrograph of the GO shows superposition of the graphene sheets, in a random distribution that produce a wrinkled surface. The alignment of the GO is probably due to the van deer Waals interaction between graphene sheets and the carbon sensor surface [22]. Finally in Fig. 2c and 2F the micrograph of the composite formed by the PPy-GO swowsare successfully synthesized. A careful analysis of the structure of the PPy-GO nanocomposites demonstrated some typical interresting characteristics to be mentioned. First, the PPy-GO nanocomposites co-electrodeposited were highly porous and consisted of two-dimensional composite nanosheets, which interconnected with each other. In addition, a notable PPy coating on the surface of the two-dimensional GO was carried out through an electrochemical approach, providing excellent functionalities.

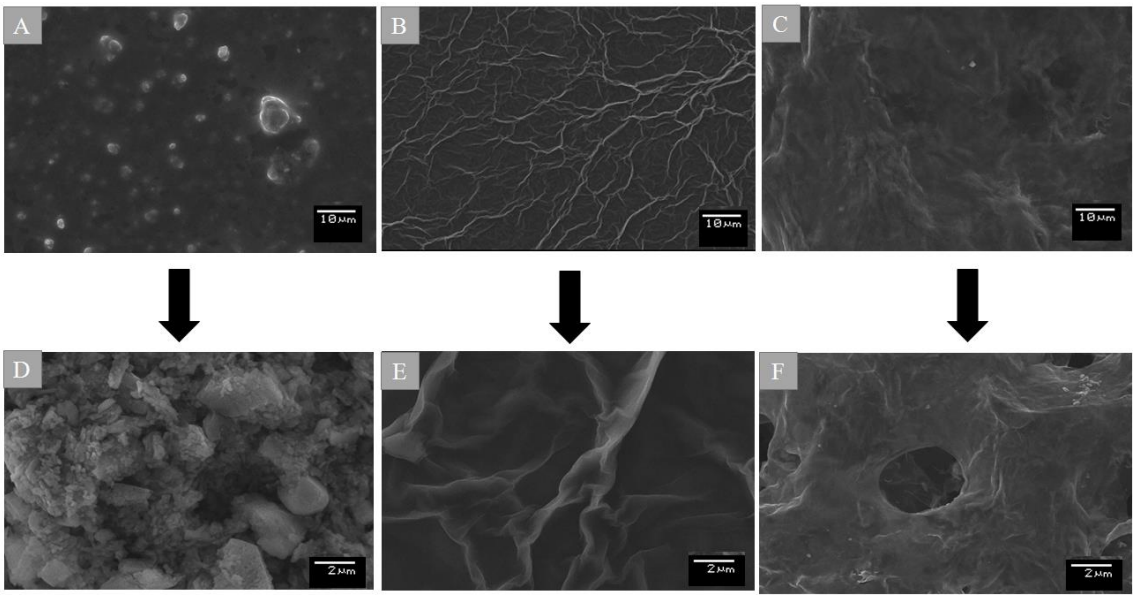

Fig 2. SEM images (A;D) PPy; (B;E) GO; (C;F) Ppy@GO in diferent increments.

The electron diffusion study of the PPy-GO/GCE was investigated submitting the electrode to different scan rates. As can see in Fig. 3, the voltammograms registered in a $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ (0.005mol L^{-1}) exhibit a proportional increase in both cathodic and anodic peak currents (Ipa and Ipc, respectively) according to the scan rate (10 – 150 mVs^{-1}). The Ipa and Ipc were directly proportional to the square root of scan rates (Fig. 3 - inset) with the following linear regression equations: Ipa (µA) 29,823 x – 37,537 (r = 0.99) and Ipc (µA) y = -26,567 x + 22,477 (r = 0.99). These results suggest that the reactions on the sensor interface were controlled by mass transport (surface diffusion) [23].

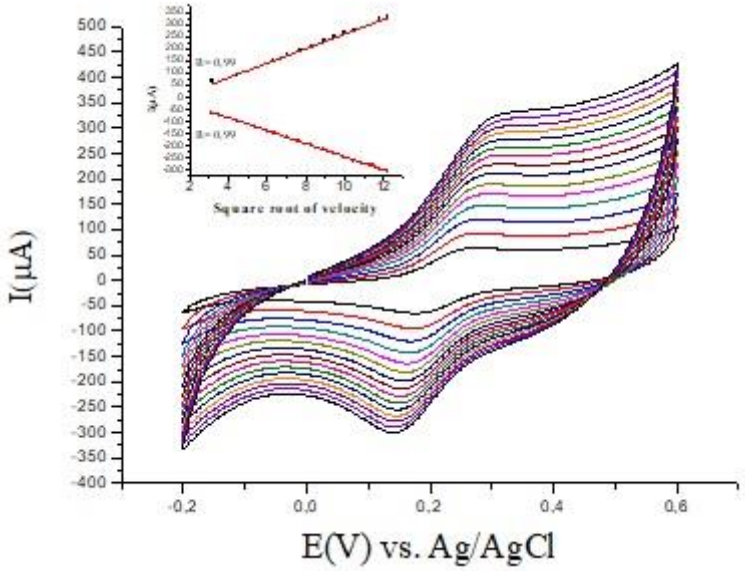

Fig. 3. Voltammetric profile of the PPy-GO/GCE under different scan rates (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 mVs^{-1}) (inset: plots of the Ipa and Ipc vs. square roots of the scan rates). All the measurements were performed in $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ (0.005 mol L^{-1}) prepared in KCl solution (0.1 mol L^{-1}).

The film stability was evaluated by submitting the PPy-GO\GCE to 20 voltammetric cycles in a potential window varying from -0.2 to 0.6 V at 50 mVs^{-1} of scan rate. According to Fig. 4, the redox peaks were practically constant during all scanning. The coefficient of variation was about 0.75 % for the anodic and cathodic peaks, indicating that the PPy-GO\GCE film presented a high stability. This could be attributed the number of hydroxy, carboxy and epoxy groups of GO, the pyrrole molecule is readily adsorbed on the surface of GO by electrostatic adhesion and π interactions. After in situ chemical polymerization, the PPy uniformly covered onto the surface of GO sheets and obstructed the aggregations of the GO. In fig. 4 is show a homogeneity in the areas presented by the successive voltammograms performed, these results corroborate with the high stability evidenced by the anodic and cathode peaks.

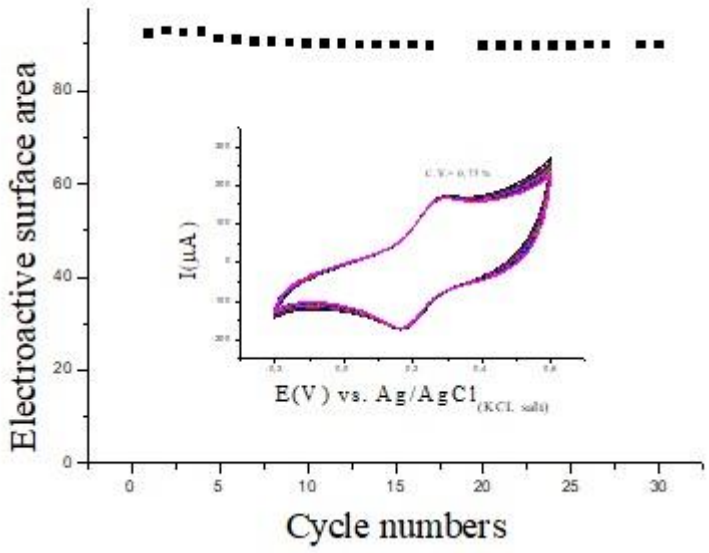

Fig. 4. Successive cyclic voltammetries and electroative surface area of the Ppy-GO/GCE

3.1.2 Chemical characterization by FTIR-ATR

FTIR-ATR is a good technique for study the surface properties, since that allows an infrared beam penetration in depth of around 0.5 - 3 μm, depending on the ATR crystal material. Here, it was used the Ge crystal, in which the evanescent wave penetrates ~ 0.65 μm at surface. Analyses of FTI- ATR spectra confirmed the presence of PPy and GO as a nanocomposite according to **Fig. 5**. In curve I and II, it were found the peaks at 1159 cm^{-1} 847 cm^{-1} that were attributed to C–H wagging, respectively [24, 25]. It was also observed, in curve I, the characteristic peak at 1635 cm^{-1}, representing the C=N bonds of PPy [19]. Additionally, small peaks at 3400 cm^{-1} corresponding to the N–H stretching vibrations of PPy, these peaks were also previously described for PPy [25, 26]. In curve II e III, peaks at 3466 cm^{-1} and 3418 cm^{-1} were respectively attributed to O-H stretching vibrations of GO [24, 25]. The peak at 1652 cm^{-1} and 1151 cm^{-1} were attributed to C=O and C-O stretching, respectively, also indicating the GO presence. It was assigned to C−H and C-C backbone stretching of PPy, the peak at 626 cm−1 (curve III), as probable indicative that the PPy was successfully polymerized on the GO.

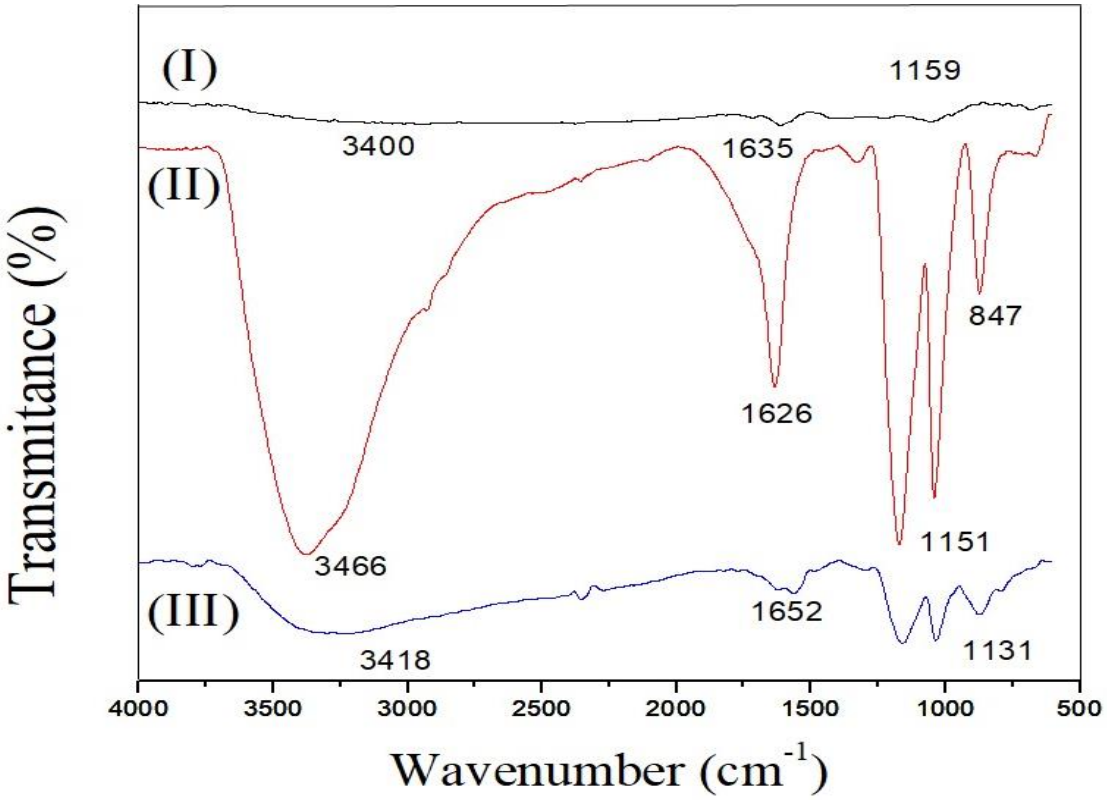

Fig. 5. ATR FT-IR spectra of the GO modified (curve I) and PPy modified (curve II) and PPy-GO (curve III).

3.2 Experimental optimization

It was optimized the SA concentration as function available carboxyl groups of PPy-GO. Initially, the nanocomposite was activated using chains by reaction with an aqueous solution of N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS). EDC/NHS is a very common coupling method for creation of amide bonds [26]. These coupling agents have high solubility in aqueous media, which allows the reaction to proceed in the absence of organic solvents. In this reaction, the EDC interacts with the carboxylic groups for the formation of reactive esters. However, the attack of the amine on this intermediate complex may become slow and hydrolyze in aqueous solutions. In this way, it is necessary the assistance of the NHS for the formation of more reactive esters and prone to the formation of stable amide bonds [26, 27].

After chemical activation with EDC/NHS, the SA was immobilized on the nanocomposite, probably on the carboxyl group of GO. SA is a 52.8 kDa protein purified

from the bacterium *Streptomyces avidinii* [28]. In this study, a proportional increase in the amplitude of the peak currents in relation to SA concentrations was found until reach a maximal response at 30 µg L^{-1}(Fig 6).

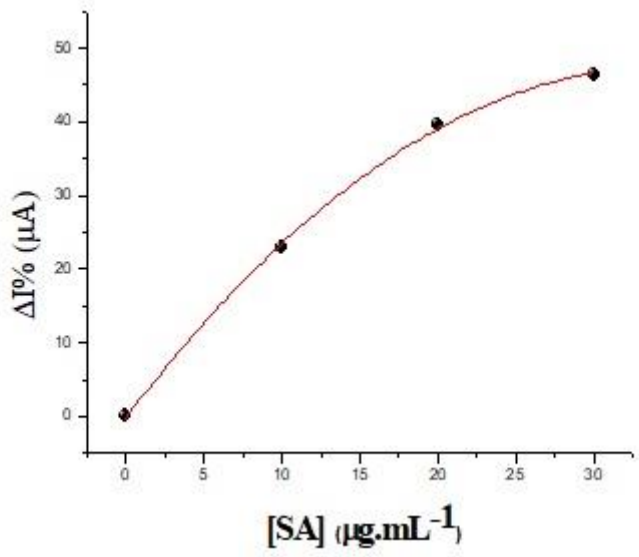

Fig. 6. Plots of the Ipa vs. different concentrations of SA.

Strategies using the SA-biotin complex have been applied to several immunoassays [28], due to their high affinity constant that is the strongest noncovalent biological interaction known, with dissociation constant (K_d) in the femtomolar range [29]. The relationship for these molecules assures that for one molecule of SA four Biotin molecules are bound, allowing increase on immunosensor sensitivity. It also investigated an optimal concentration of HCV antigen (HCVcAg) biotinylated to be immobilized on the platform. Xiao et al (2014) [30] in their studies, found that the optimum concentration for the immobilization of HCVcAg in the HS layer, as well as for the formation of the antibody antigen complex is 100 µg. mL^{-1}. So, it concentrations was chose to reamin experiments (Fig. 7)

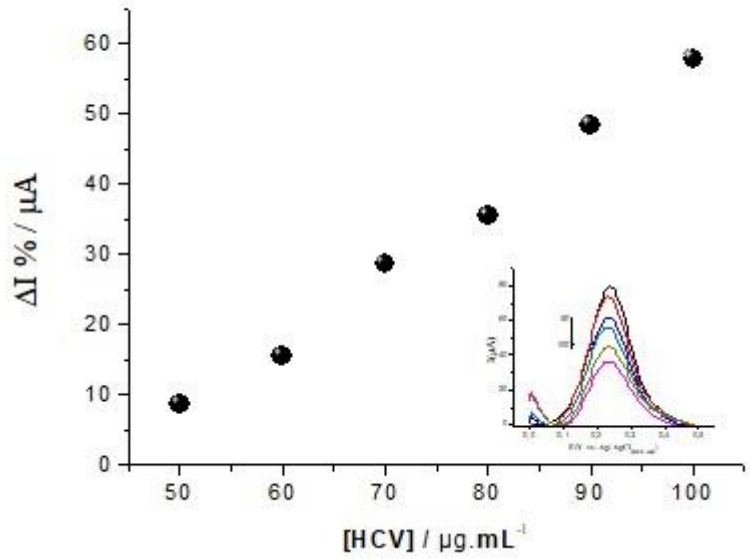

Fig 7. Plots of the Ipa vs. different concentrations of antigen HCV (Inset: Square wave voltammetry profile of the different concentrations of antigen HCV (50, 60, 70, 80, 90 and 100 μg.mL^{-1}).

3.4 Electrochemical characterization

The Fig. 8 shows electrochemical behavior in each step of immunosensor preparation. It was possible to observe the PPy-GO film (curve I) formed on the surface of the GCE, which was activated with EDC-NHS, thus enabling the immobilization of SA II). The immobilization of HCV Antigen was incubated on to the electrode surface, there was an obvious decrease of the redox current peaks (curve III) demonstrating that HCVcAg biotinylated were immobilized on the electrode surface. The reason for decrease of peak current is that HCV proteins due to their insulating nature hinder the transmission of electrons toward the electrode surface. Afterwards, glycine was added on the electrode surface to block possible non-specific binding, improving the analytical specificity (curve IV). Finally, when Anti-HCV was captured, the peak current decreased again (curve V), indicating the successful capture of Anti-HCV and the formation of immunocomplex.

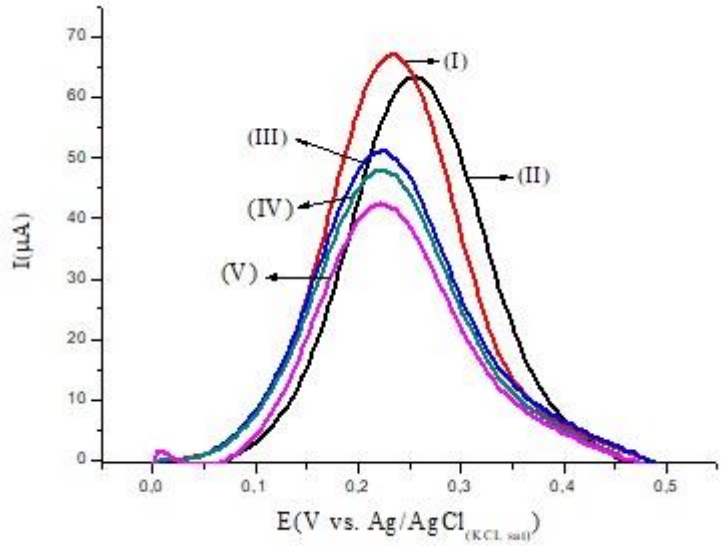

Fig 8. SWV of the stages of the immunosensor construction (I) PPy-GO/GCE, (II) STV/PPy-GO/GCE, (III) HCVcAg/STV/PPy-GO/GCE, (IV) Glycine/HCVcAg/STV/PPy-GO/GCE, (V) Anti-HCV/Glycine /HCVcAg/STV/PPy-GO/GCE

3.3 Calibration curve of the Anti-HCV

The calibration curves obtained as response of different Anti-HCV concentrations are shown in Fig. 9. The amperometric responses were generated by the amplitude of anodic peaks. The plateau was achieved at 14 ng mL^{-1}. The data adjusted by linear regression equation Y (I/) = 5.17 X +19.89, showed a correlation coefficient of 0.981 ($p < 0,05$). The limit of detection was 1.63 ng mL^{-1} according to IUPAC (LOD = 3 sd/slope). Sensitivity is an important parameter that influences the performance of an immunosensor in practical application, herein the LOD allowed the use of immunosensor in real samples, to distinguish anti-HCV serum samples of infected patients. This found LOD was similar to ELISA 10 $ng/mL^{-1)}$ and in comparison with previous studies, this immunosensor showed a good sensitivity as observed in Table 1.

	Transdutor	Faixa linear de detecção	Limite de detecção
ZHAO & LIU (2016) [31]	Eletrochemical	10-80 ng/mL^{-1}	750 pg/mL^{-1}
ARONOFF-SPENCER et al. (2016) [32]	Eletrochemical	0,1-1000 ng/mL^{-1}	10 ng/mL^{-1}
MORAES et al. 2013 [33]	Eletrochemical	1 - 0.2 µg.mL^{-1}	10 ng/mL^{-1}

Table 1: comparison between immunosensors described in the literature.

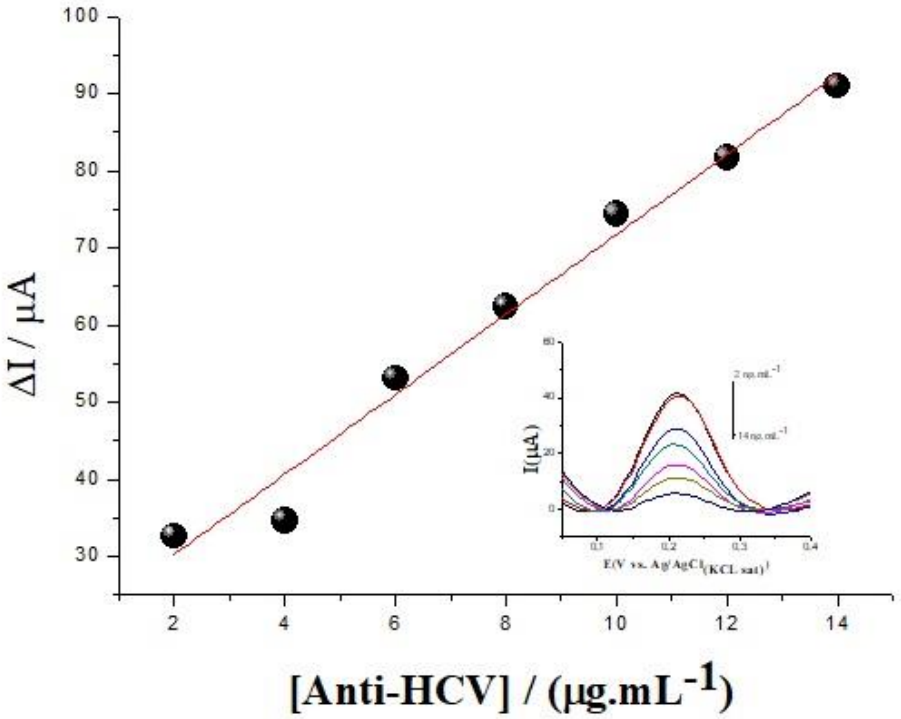

Fig. 9. Analytical curve of the Ppy-GO nanoelectrode for different Anti-HCV concentrations (2-14 ng.mL^{-1}) obtained by SWV measurements in $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ (0.005 mol.L^{-1}) prepared in KCl (0.1 mol L^{-1}) (Inset: SWV of the 2-14 ng mL^{-1})

The immunosensor was checked against spiked serum samples submitting it to an aliquot of 5 µL during 30 min, in a moist chamber at room temperature (~23 ºC). The response of the sensor platform was obtained by using the percentage decrease of current (IΔ %) of the SWVs measurements in 5 mM K3 [Fe (CN) $_6$]/ K_4 [Fe (CN) $_6$] prepared in 0.1 KCl. According to curve I, it was observed that difference of peak currents from the blank gradually increase with the anti-HCV concentrations, conversely to the non-spiked blood that

was practically constant in all negative blood samples (curve II). This result suggests that the immunosensor achieve the specificity to recognize anti-HCV in the complex medium / matrix, containing several proteins, lipids, cells, debris, etc (Zhong et al., 2015).

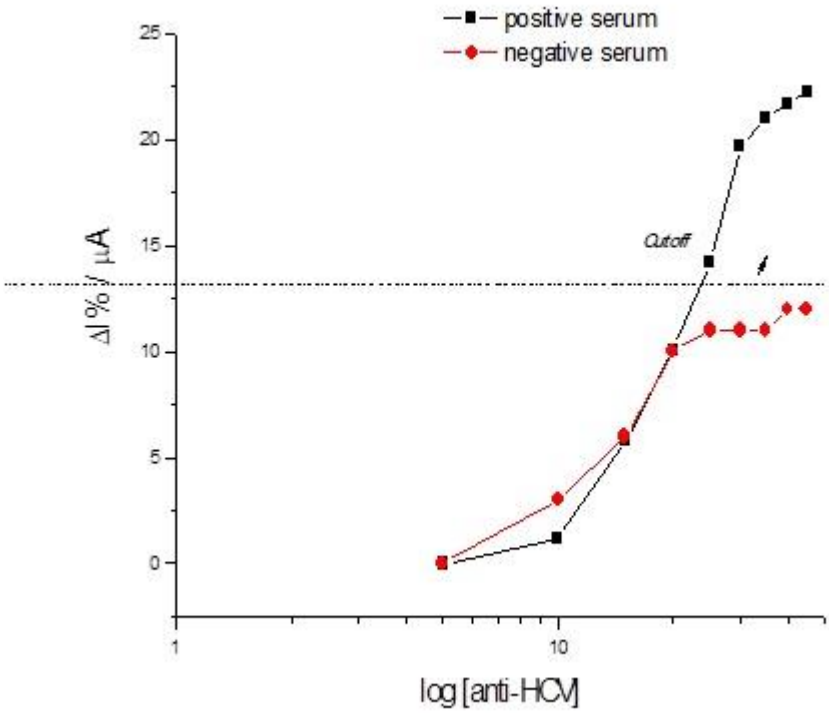

Fig. 10. Analytical curve of response to antibodies present in positive (curve I) and negative (curve II) anti-HCV under optimal experimental conditions.

4. Conclusions

The high performance seems probably due to the synergic PPy-GO effect that enabled great amount of immobilized antigen and all owed an increase on the electron transfer that leaded to a high diagnostic sensitivity.

A label-free immunosensor was developed to detect HCV antibodies. The platform used was shown to be sensitive and stable. Anti-HCV is a valuable marker for use in the blood banks, the objective of screening donors, since it indicates a prior contact with the virus at any time in life.

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References.

[1] S. Zhou, S. Cao, G. Ma, T. Ding, J. Mu, W. Han, D. Sun, C. Chen, Recombinant streptavidin fusion proteins as signal reporters in rapid test of human hepatitis C virus infection, J Clin Lab Anal 29 (2018) 1-7.

[2] P. Zeng, P. Hou, C. J. Jing, C. Z. Huang, Highly sensitive detection of hepatitis C virus DNA by using a one-donor-four-acceptors FRET probe, Talanta 185 (2018) 118–122.

[3] A. Llibre, Y. Shimakawa, E. Mottez, S. H. Ainsworth,T. Buivan, R. Firth, E. Harrison, A. R. Rosenberg, J. Meritet, A. Fontanet, P. Castan, A. Madejón, M. Laverick, A. Glass, R. Viana, S. Pol, C. P. McClure, W. L. Irving, G. Miele, M. L. Albert, D. Duffy, Development and clinical validation of the Genedrive point-of-care test for qualitative detection of hepatitis C virus, Hepatology 1 (2018) 1-8.

[4] L. M. Villar, H. M. Cruz, J. R. Barbosa, C. S. Bezerra, M. M. Portilho, L. P. Scalioni, Update on hepatitis B and C virus diagnosis, World J Virol 12 (2015) 323-342.

[5] S. M. Shawky, A. M. Awad, W. Allam, M. H. Alkordi, S. F. EL-Khamisy, Gold aggregating gold: A novel nanoparticle biosensor approach for the direct quantification of hepatitis C virus RNA in clinical samples, Biosensors and Bioelectronics 92 (2017) 349–356.

[6] D. G. Fisher, K. L. Hess, E. Erlyana, G. L. Reynolds, C. A. Cummins, T. A. Alonzo, Comparison of Rapid Point-of-Care Tests for Detection of Antibodies to Hepatitis C Virus, Ofid 1 (2015) 1-6.

[7] J. H. Ryu, B.S. M. Kwon, J. Moon, M. Hwang, J. Lee, K. Park, S. J. Yun, H. J. Bae, A. Choi, H. Lee, B. Jung, J. Jeong, K. Han, Y. Kim, E. Oh, Development of a Rapid Automated Fluorescent Lateral Flow Immunoassay to Detect Hepatitis B Surface Antigen (HBsAg), Antibody to HBsAg, and Antibody to Hepatitis C, Ann Lab Med 38 (2018) 578-584

[8] D. Ananya, R. Vimala, Natural and synthetic polymer for graphene oxide mediatedanticancer drug delivery—A comparative study, International Journal of Biological Macromolecules 107 (2018) 2320–2333.

[9] L. Chen, J. Moon, X. Ma, L. Zhang, Q. Chen, L. Chen, R. Peng, P. Si, J. Feng, Y. Li, J. Lou, L. Ci, High performance graphene oxide nanofiltration membrane prepared by electrospraying for wastewater purification, Carbon 130 (2018) 487- 494.

[10] X. Huang, T. Leng, T. Georgiou, J. Abraham, R. R. Nair, K. S. Novoselov, Z. Hu, Graphene Oxide Dielectric Permittivity at GHz and Its Applications for Wireless Humidity Sensing, Nature 8 (2018) 1-7.

[11] W. K. Chee, H. N. Lim, N. M. Huang, I. Harrison, Nanocomposites of graphene/polymers: a review, RSC Adv 5 (2015) 68014–68051.

[12] S. Pourbeyram, P. Kheyri, Graphene/polypyrrole nanofiber prepared by simple one step green method for electrochemical supercapacitors, Synthetic Metals 238 (2018) 22–27.

[13] Kh. Ghanbari, S. Bonyadi, An electrochemical sensor based on reduced graphene oxide decorated with polypyrrole nanofibers and zinc oxide–copper oxide p–n junction heterostructures for the simultaneous voltammetric determination of ascorbic acid, dopamine, paracetamol, and tryptophan, New J. Chem. 42 (2018) 8512—8523.

[14] C. Ott, M. D. Raicopol, C. Andronescu, E. Vasile, A. Hanganu, A. Pruna, L. Pilan, Functionalized polypyrrole/sulfonated graphene nanocomposites: Improved biosensing platforms through aryl diazonium electrochemistry, Synthetic Metals 235 (2018) 20–28.

[15] P. M. Nia, W. P. Meng, F. Lorestani, M.R. Mahmoudian, Y. Alias, Electrodeposition of copper oxide/polypyrrole/reduced grapheneoxide as a nonenzymatic glucose biossensor, Sensors and Actuators B 209 (2015) 100–108.

[16] H. Dai, N. Wang, D. Wang, H. Ma, M. Lin. An electrochemical sensor based on phytic acid functionalized polypyrrole/graphene oxide nanocomposites for simultaneous determination of Cd(II) and Pb(II), Chemical Engineering Journal 299 (2016) 150–155.

[17] S. Liu, J. Wang, J. Zeng, J. Ou, Z. Li, X. Liu, S. Yang, “Green” electrochemical synthesis of Pt/graphene sheet nanocomposite film and its electrocatalytic property, Journal of Power Sources 195 (2010) 4628–4633.

[18] C. Li, H. Bai, G. Shi, Conducting polymer nanomaterials: electrosynthesis and applications, Chem. Soc. Ver. 38 (2009) 2149–2496.

[19] Z. Cai, H. Xiong, Z. Zhu, H. Huang, L. Li, Y. Huang, X. Yu, Electrochemical synthesis of graphene/polypyrrole nanotube composites for multifunctional applications, Synthetic Metals 227 (2017) 100–105.

[20] D. R. Dreyer, S. Park, C. W. Bielawski, S. R. Ruoff, Harnessing the chemistry of graphene oxide, Chem. Soc. Rev. 39 (2010) 228 - 240.

[21] A Afzal, F. A. Abuilaiwi, A. Habib, M. Awais, S. B. Waje, M.A. Atieh, Polypyrrole /carbon nanotube supercapacitors: Technological advances and challenges, Journal of Power Sources 352 (2017) 174 – 186.

[22] R. Moosaei, M. Sharif, A. Ramezannezhad, Enhancement of tensile, electrical and thermal properties of epoxy nanocomposites through chemical hybridization of polypyrrole and graphene oxide, Polymer Testing 60 (2017) 173 – 186.

[23] X. Kanga, J. Wanga, H. Wua, I. A. Aksayc, J. Liua, Y. Lin, Glucose Oxidase–graphene–chitosan modified electrode for direct electrochemistry and glucose sensing, Biosensors and Bioelectronics 25 (2009) 901–905

[24] C. Bora, S. K. Dolui, Fabrication of polypyrrole/graphene oxide nanocomposites by liquid/liquid interfacial polymerization and evaluation of their optical, electrical and electrochemical properties, Polymer 53 (2012) 923-932.

[25] C. Zhu, J. Zhai, D. Wen, S. Dong, Graphene oxide/polypyrrole nanocomposites: one-step electrochemical doping, coating and synergistic effect for energy storage, J. Mater. Chem. **22** (2012) 6300-6306.

[26] S. Sam, L. Touahir, J. S. Andresa, P. Allongue, J.-N. Chazalviel, A. C. Gouget-Laemmel, C. Henry de Villeneuve, A. Moraillon, F. Ozanam, N. Gabouze, S. Djebbar, Semiquantitative Study of the EDC/NHS Activation of Acid Terminal Groups at Modified Porous Silicon Surfaces, Langmuir 26 (2010), 809–814.

[27] Z. Guler, A. S. Sarac, Electrochemical impedance and spectroscopy study of the EDC/NHS activation of the carboxyl groups on poly(!-caprolactone)/poly(m-anthranilic acid) nanofibers, Express Polymer Letters 10 (2016) 96–110.

[28] Y.-P. Wu, C. Y. Chew, T.-N. Li, T.-H. Chung, E-H. Chang, C. H. Lam, K-T. Tan, Target-activated streptavidin–biotin controlled binding probe, Chem. Sci. 9 (2018) 770 – 776.

[29] N. Bansal, Z. Zheng, L.F. Song, J. Pei, K. M. Merz, Jr., The Role of the Active Site Flap in Streptavidin/Biotin Complex Formation, J. Am. Chem. Soc. 140 (2018) 5434−5446.

[30] R. Xiao, Z. Rong, S. Chen, W. Chen, S. Wang, Optic fiber-based immunosensor for the rapid and sensitive detection of hepatitis C virus in serum, RSC Adv. 4 (2014) 36125–36130.

[31] C. Zhao, X. Liu, A portable paper-based microfluidic platform for multiplexed electrochemical detection of human immunodeficiency virus and hepatitis C virus antibodies in serum, Biomicrofluidics 10 (2016) –11.

[32] E. Aronoff-Spencer, A. G.Venkatesh, A. Sun, H. Brickne, D. Looney, D. A. Hall, Detection of Hepatitis C core antibody by dual-affinity yeast chimera and smartphone-based electrochemical sensing, Biosensors and Bioelectronics 86 (2016) 690–696.

[33] M. L. Moraes, L. R. Lima, R. R. Silva, M. Cavicchioli, S. J. L. Ribeiro, Immunosensor Based on Immobilization of Antigenic Peptide NS5A-1 from HCV and Silk Fibroin in Nanostructured Films, Langmuir 29 (2013) 3829−3834

[34] C. V. Uliana, C. S. Riccardi, H. Yamanaka, Diagnostic tests for hepatitis C: recent trends in electrochemical immunosensor and genosensor analysis, World J Gastroenterol 20 (2014) 15476 - 15491.

5.2 ARTIGO 2 - Immunoassay for the detection of biomarker for Hepatitis C virus based on GO-Pth nanocomposite

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Label-free electrochemical immunosensor with CTAB intercalated graphene for Hepatitis C virus antibodies detection

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Abstract

A new label-free immunosensor containing cetyltrimethylammonium bromide (CTAB) intercalated graphene oxide (GO) on the polythiophene (PTh) film one-step electrosynthetized is described for detection of hepatitis C antibodies (Anti-HCV). This electroactive film also acted as a substrate for immobilization of the bio-recognition element. The ability of the biosensor to bind Anti-HCV was monitored by square wave voltammetry (SWV) through decrease on the anodic peaks. The intercalation of GO nanosheets between CTAB and PTh was very stable achieving at 30 cycles a variation coefficient approximately of 0.65% for redox peaks, and also observed a diffusion-controlled process and electrochemical reversibility. The immunosensor showed a response linear range from 0.2 – to 8 ng mL^{-1} and good reproducibility (square R equal to 0.993, low relative error <1%) and a limit of detection at 0.07 ng mL^{-1} of Anti-HCV allowing clinical range. This approach shows as potential for development of a practical and reliable immunosensor for HCV diagnostic in chronic stages, being as also possible to monitor other HCV biomarker using this technology.

Keywords: polythiophene; graphene oxide; label-free detection; hepatitis C; amperometric immunosensor.

1. Introduction

Viral hepatitis is a major public health concern, infecting millions of people worldwide. According to the World Health Organization, over 250 million people are currently infected with Hepatitis B virus (HBV) and more than 70 million with Hepatitis C virus (HCV) (Valva et al., 2016). Some infections subsequently lead to hepatocellular carcinoma, liver cirrhosis and fatalities among significant proportion of patients (Jefferies et al., 2018). HBV-HCV coinfection is more complex than monoinfection with HBV or HCV alone, and in co-infected cells the dominance of HCV over HBV replication is observed (Mavilia and Wu, 2018). Management and treatment of HCV patients has changed rapidly after introducing the antiviral medicines like the sofosbuvir, daclatasvir and the sofosbuvir/ledipasvir combination that can achieve approximately cure rates above 95% (Kawagishi et al., 2017; Sun et al., 2018). Although HCV treatment has a promise of a great successful in nowadays, the access to diagnosis and treatment is still low. Many infected individuals do not know that are a positive-host virus, due to the HCV is usually asymptomatic (Shawky et al., 2017). Reliable, practice, sensitive and screening testings to the hepatitis diagnostic are desirable for affordable managements, control of spreading and minimizing the symptoms, especially in poor and limited self-constraint countries.

HCV diagnostic methods mostly used today are enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence immunoassay (ECLIA) (Khan et al., 2017), beside recombinant immunoblot assay (RIBA) as confirmatory assay. The ribonucleic acid (RNA)-polymerase chain reaction molecular testings to determine the HCV genotype and quantify HCV RNA are the gold standard, but are more sensible in high viremia or acute phase and also involve relatively high-cost, time-consuming procedures (Lima et al., 2018). Thus, there is potential for the use of simpler and cheaper tests to confirm HCV infection in different clinical settings. Alternatively, there is a great interest in electrochemical immunosensors, owing to their ability to perform quantitative analysis with fewer background noises and lower costs (Uliana et al., 2014; Bhardwaj et al., 2019).

HVC is a positive-sense single-stranded RNA virus belong to the *flaviviridae* family (Khan et al., 2014). The genome includes a single open reading frame that encodes a precursor polyprotein, with three structural proteins (the core protein and two envelope proteins E1 and E2), viroporin p7 and six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Nakano et al., 2018). Quantitative Antibody Responses to Structural (Core) and Nonstructural (NS3, NS4, and NS5) Hepatitis C Virus Proteins (Khan et al., 2017)

The HCV core protein is a conserved the six different genotypes it produces multiple changes in gene transcription, signal transduction, immune presentation, and cell-cycle regulation (Sun et al., 2018). Although there are many candidates as biomarker for HCV diagnosis, anti-HCV antibody testing and HCV RNA testing are used to diagnose acute and chronic hepatitis C, respectively (Singhal et al., 2017).

In recent years, carbon allotropes nanomaterials are widely applied in biosensing systems, especially in bioassays, due to their excellent electrical properties (Trindade and Dutra, 2018). Among them, graphene oxide (GO) stands out due to it is because of its remarkable electrical, thermal and mechanical properties (Wang et al., 2019). GO is oxidized derivative of graphene, possessing both the graphene-like 2D carbon sheet structure and various oxygen functional groups such as epoxy, carboxyl, carbonyl, and hydroxyl groups. The coexistence of 2D single layer sp2 carbon sheet structure and oxygen functional groups in GO endows its useful physicochemical properties including amphiphilicity, stability in aqueous solutions, strong adsorption of certain molecules onto the GO plane through π−π stacking and hydrogen bonding, and facile surface modifications (Kim et al.,2016; Wang et al., 2017). GO structure consists of conjugated domains Sp_2 interspersed with polar oxygenated domains, and these properties endow GO with the ability to pass both ionic and nonionic bonding with a wide range of molecules. (Wang et al., 2017). Modification of the GO sheets through conductive polymers assures a greater fixation on the sensor surface avoiding possible leaching (Chung et al., 2012).

Polythiophene (PTh), one of the most important materials in the family of the conducting polymers, has attracted intense interest due to its promising electric, electrochemical and optical properties (Shamsayei et al., 2016). Thiophene is a five membered heterocyclic aromatic compound. Its chemical stability, easy synthesis and easy processing make its derivatives among one of the most studied organic compounds (Saeed et al., 2017). In this study, a nanocomposite based on graphene and polythiophene oxide was synthesized in a single step using as a cationic ionic liquid, Cetrimonium bromide (CTAB) as solvent in order to obtain a clean chemical synthesis with little residue generation. The method described herein involves one-step preparation process and represents an advance in the production of immunosensor testing.

2. Material and methods

2.1 Reagents and serum samples

Th monomers (98%, v/v), GO in aqueous solution (0.2 µg mL^{-1}), Streptavidin (SA), glycine, potassium ferrocyanide ($K_4Fe(CN)_6$), potassium ferricyanide ($K_3Fe(CN)_6$), Dimethyl sulfoxide (DMSO), Cetrimonium bromide CTAB), N-hydroxysuccinimide (NHS) and N-ethyl-N′-(3-dimethylami-nopropyl) carbodiimide (EDC) were obtained from Sigma Aldrich (St.Louis, USA).The biotynilated HCV antigen (HCVcAg) and monoclonal human anti-HCV were purchased from Abcam (Cambridge, UK).

The phosphate buffered saline (PBS) (0.01 mol L^{-1}, pH 7.4) was used in all dilutions of protein samples. The ultrapure water was obtained from a water purification system Milli-Q (Billerica, USA) (18MΩ) and it was utilized to prepare all solutions. All chemicals used in the study were of analytical reagent grade purity.

Serum samples were extracted from healthy individuals using a Vacutainer tubes and centrifuged at 6.000 RPM. Afterwards, a pool of ten samples was obtaining by homogenizing in a vortex mixer and spiked with Anti-HCV diluted in PBS. Negative samples were carefully prepared with the same volume of PBS, in order that the signal increment in response to incubations were exclusively due to the anti-HBc and the matriz effect was investigated.

2.2 Apparatus

The electrochemical studies were performed by using a portable potentiostat/galvanostat Ivium Compact Stat (Eindhoven, NLD) connected to a microcomputer and controlled by Ivium Soft software. It was used a three electrode system, comprising a gold electrode (GE) (Ø = 3 mm) as the working electrode, a helical platinum wire as counter electrode and $Ag/AgCl_{(KCl\ saturated)}$ as the reference electrode. Electrochemical analyses were performed in an electrochemical cell (10mL) conducted at room temperature (~ 24 °C).

2.3 Electrochemical characterization

The electrochemical analyzes were performed by using the cyclic voltammetry technique (CV) with a potential window of -0.2 to 0.6 V, at 0.5 mV s^{-1} scan rate. Analyzes performed by square wave voltammetry (SWV) were recorded between 0.0 and 0.5 V at 10Hz with pulse amplitude of 10 mV and step potential of 2.5 mV.

2.4 Preparation of the PTh/GO/GE

Prior to modification (Fig. 1 A), the GE was polished with alumina slurry (1 and 0.3 µm) for 5 minutes and rinsed with ultrapure water. Initially, the GO was dispersed in 0.2 mg mL^{-1} CTAB aqueous solution and sonicated (14KHz) at room temperature for 30 min to get single-sheet dispersion.

Afterwards, the GE was immersed in a mix solution of the TH monomers (0.8 mmol. L^{-1}) prepared in DMSO solution, GO (0.2 mg mL^{-1}) and CTAB and submitted to electropolymerization procedure. It was performed in a single one-step of synthesis by using the cyclic voltammetry technique in a potential window of -0.8 to 0.8 V, by 50 cycles at 100 mV s^{-1} scan rate.

2.5 HCV antigen immobilization

The functional groups derived of the PTh-CTAB-GO nanocomposite were activated by incubation with EDC/NHS solution (0.02/0.05 mol L^{-1}) prepared in deionized water during 1 h at room temperature. Then, the SA (SA) (40 µg mL^{-1}) was covalently immobilized on the nanocomposite film in order to conjugate the biotinylated HCVcAg on the sensor surface. An aliquot (5 µL) of the HCV solution (100 µg mL^{-1}) was dropped on to the electrode surface and incubated for 1 h at room temperature. Non-specific bindings were blocked by incubating the GE surface in a solution of glycine (0.05 mol L^{-1}), prepared in ultra-pure water, during 40min.

2.6 Analytical responses

The label-free electrochemical immunoassay was based on the diffusion of $[Fe(CN)6]^{3/4-}$ redox probe through PTh-CTAB-GO nanocomposite during the antigen-antibody recognition event. It was monitored by square wave voltammetry (SWV) measurements. These measures were recorded between 0.0 and 0.5 V at 10Hz with pulse amplitude of 10 mV and step potential of 2.5 mV. The detection of antibodies against HCV was standardized by using the perceptual decrease of current (ΔI %) in the SWV measurements of the HCV-modified electrode before (blank) and after incubations with different antibodies concentrations). All measurements were performed in triplicate.

3. Results and discussion

3.1 Electrosynthesis of the PTh-CTAB-GO nanocomposite

The impact the synthesis method plays on the electrical conductivity, chemical and thermal stability of polymers is well-known (Gracia and Mecerreyes, 2013; Riess, 2000). Herein, two methods of synthesis, forming 3D structures, the CV and drop-casting approaches have experimented (Fourati et al., 2016). The one-step cyclic voltammetry method by using a mixture containing Th monomers, GO dispersed in CTAB, showed better electrochemical profile compared with layer-by-layer achieved by drop casting technique as shown by two synthesis methods in Fig. 1(A). It was observed on the SWV plot, higher anodic peaks probably due to the better CTAB intercalation with GO, which the positively charged ammonium ions of the CTAB head and the negatively charged carboxyl groups of GO are linked through ionic interactions (Vaghri et al., 2018). In addition, this good electron transfer can be also attributed to the stronger coupling, which owing to the π–π interaction between the nonpolar regions of GO and molecular layers of PTh (Filip et al., 2015). In Fig. 1B is illustrated a schematic representation of formation of the PTh-CTAB-GO nanocomposite in the sensor platform.

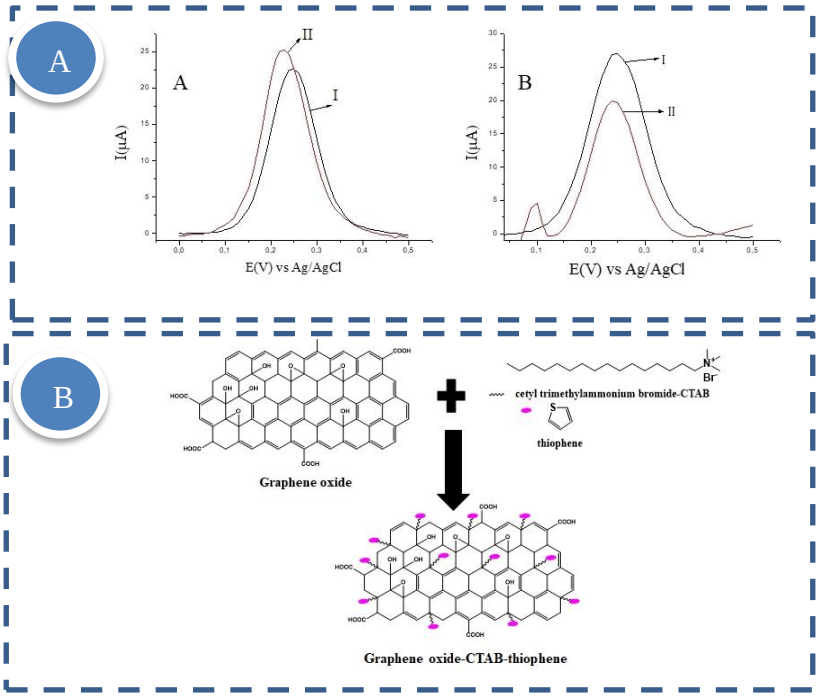

Fig. 1. (A) SWV measurements of the current (black solid line) before and after PTh-CTAB-GO nanocomposite preparation (red solid line) obtained by (a) Cyclic voltammetry electropolymerization; (b) Drop-casting. **(B)** Illustration of the PTh-CTAB-GO synthesis.

Mechanistic studies to investigate the adsorption of diffused particles in a space bounded by the substrate and irreversibly adhering to the substrate on the sensor surface were performed by immersing the working electrode in the electrochemical cell filled with 5 mM redox probe solution $K_3Fe(CN)_6$ / $K_4Fe(CN)_6$ prepared in 100 mM KCl , varying the scan rates from 10 to 150 mV s-1, in a window potential of -0.2 - 0.6 V, (Fig. 3a). It was observed that the cathodic and anodic current peaks of the CVs (Ipc and Ipa, respectively) increased proportionally with the square root of the scan rates exhibiting square R equal to 0.999 for IpA and 0.996 for IpC (Fig. 3 b), which indicates that the process was diffusion-controlled. In addition, the potentials of the reduction-oxidation couple were practically constant with the scan rate. The non-variation of the potential with scan rate should be explained by reversible mechanism.

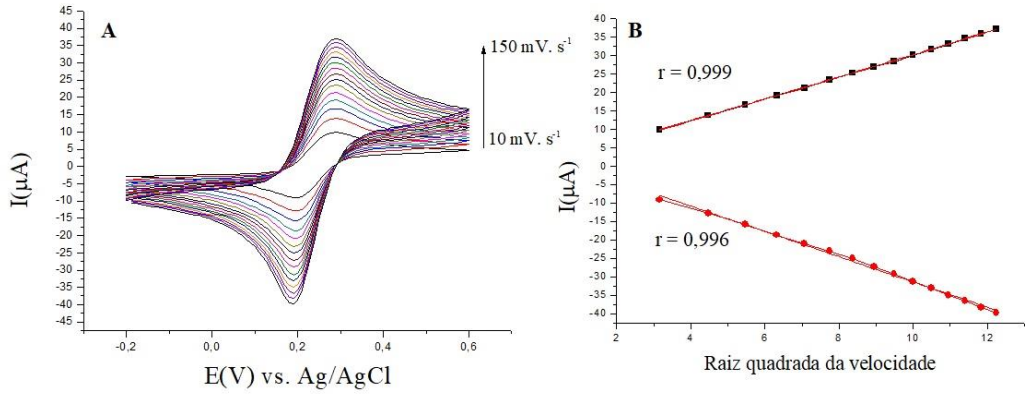

Fig. 2. A) Voltammetric profile of the film formed by Th-GO-CTAB in different scan rates (10, 20, 30, 40, 50, 60,150 mVs $^{-1}$) B) Infuence of the square root of the scan rate on the (black) cathodic and (red) anodic peak currents. Measurements performed on K_3 [Fe (CN) $_6$] / K_4 [Fe (CN) $_6$] (0.005 M) prepared in KCl buffer (0.1 M).

Electrochemical stability of the PTh-CTAB-GO film was also investigated and performed by 40 repeating of voltammetry cycles (Fig. 3). The results suggested that anodic and cathode peaks are stable during CV cycles, indicating that the electrochemical stability.

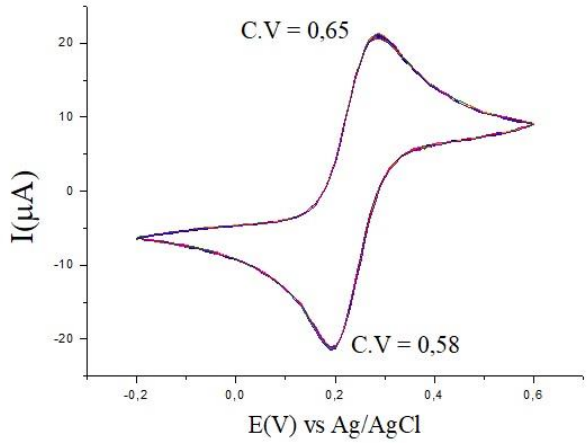

Fig. 3. Repeated cyclic voltammograms of the Thiophene-GO-CTAB film. Measurements performed on K_3 [Fe (CN) $_6$] / K_4 [Fe (CN) $_6$] (0.005 M) prepared in KCl buffer (0.1 M).

3.2 Experimental optimization

In order to obtain the best performance of immunosensor in regarding to sensitivity, maximal concentrations of the coupling agent (SA) and antigens were adopted. At first, the concentration of SA was optimized by changing concentrations of streptavidin from 10 to 50 μg.mL^{-1}. As given in Fig. 4 (A), the anodic peaks of SWV increased exponentially with the SA concentration, achieving a plateau at 40 μg.mL^{-1}, thus, this concentration was used in all remaining experiments. Afterwards, assays varying HCVcAg concentrations (10 - 60 μg.mL^{-1}) were performed on the Th-GO-CTAB/GE and was observed in plot that the plateau was obtained in 40 μg.mL^{-1}, a decrease of anodic peaks in SWV (Fig. 4(B)), a sense the chosen concentration for the accomplishment of the remain studies. The interaction between biotin and streptavidin molecules in the ratio of 4 to 1 have been explored to form the basis of many immunosensors (Dutra and Kubota, 2007; Silva et al., 2010). Analysis of crystal structures of streptavidin/biotin complex shows that high affinity results from several factors, including the formation of multiple hydrogen bonding and van der Waals interactions between biotin and protein, along with the arrangement of surface polypeptide loops that bury the biotin in the internal protein (Waner et al., 2019).

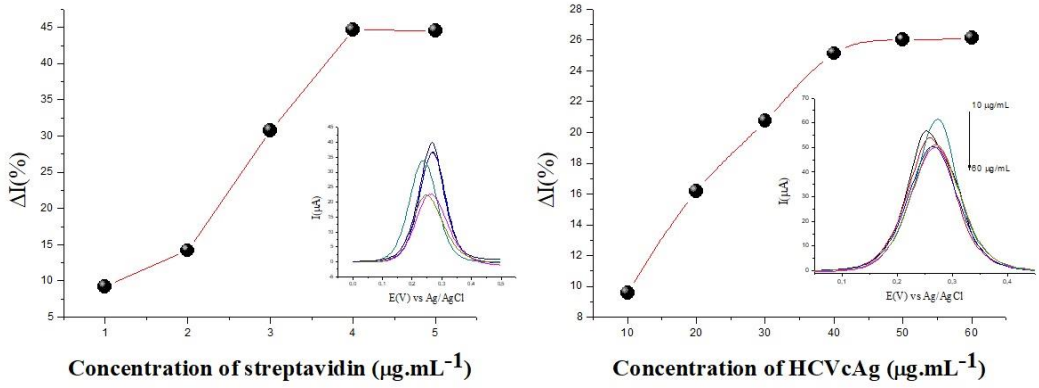

Fig. 4. (A) Current anodic peaks from SWV obtained in different concentrations of streptavidin (10 - 50 μg.mL^{-1}) (Inset: Square wave of different concentrations of streptavidin). (B) Curve with different concentrations of HCVcAg (10 - 60 μg.mL^{-1}) (Incert: Square wave of different concentrations of HCV antigen). Measurements performed on K_3 [Fe (CN) $_6$] / K_4 [Fe (CN) $_6$] (0.005 M) prepared in KCl buffer (0.1 M).

After establishing the concentration of immobilized HCV-Biot antigen on the sensor platform, glycine was used to block possible non-specific reactions with other proteins in the sample. After the blocking procedure, a reduction in the electric current was observed, decreasing the peak slightly in relation to the antigen, proving that there was blocking of the surface (Fig. 5). The presence of free amino sites in the glycine molecule provides this binding, which becomes important to avoid non-specific amide bonds with the antibody or substances present in the sample (Mahesh et al., 2018).

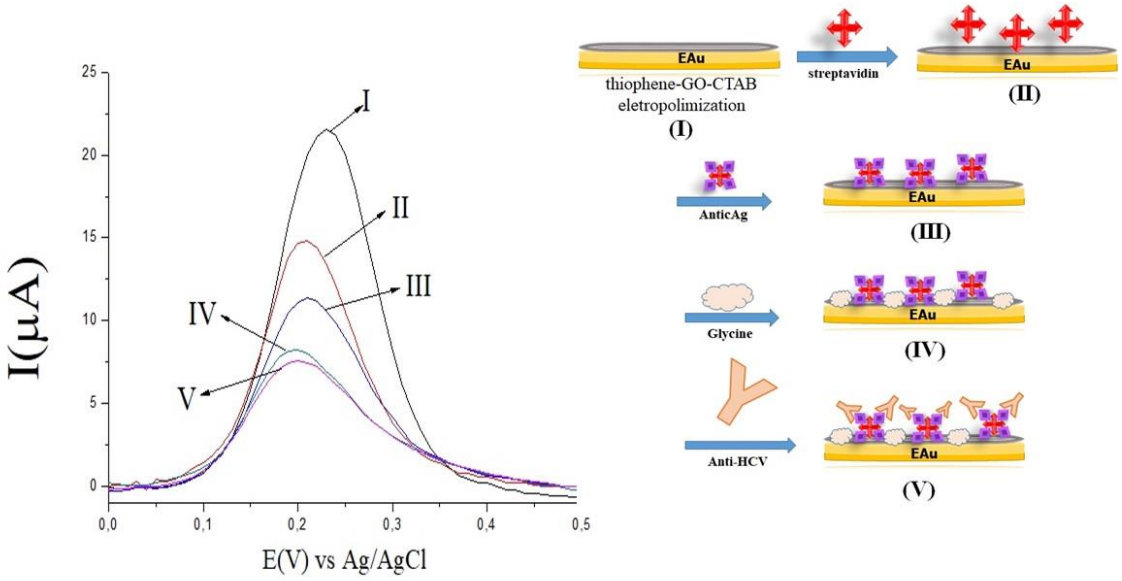

Fig. 5. Electrochemical characterization of Construction of the Immunossensor by Square Wave Technique: (I) GE modified with Thiophene-GO-CTAB, (II) STV / Thiophene-GO-CTAB / GE, (III) HCVcAg / STV / Thiophene-GO-CTAB / GE; (IV) Glicine/HCVcAg/ Thiophene-GO-CTAB / GE, (V) Anti-HCV/ Glicine/HCVcAg/ Thiophene-GO-CTAB / GE.

3.2. Response to the Anti-HCV antibodies

Under optimized conditions, the current responses of the proposed PTh-CTAB-GO based immunosensor were investigated in the presence of varying concentrations of Anti-HCV diluted in 10mM PBS (Fig 6 inset). The amperometric signals in the analyte responses decreased with increasing anti-HCV. This behavior is attributed to the insulating nature of the antibodies that by forming immunocomplexes with the antigens, they prevent the diffusion barrier (Watanabea and Hashida, 2018). The results showed a linear increase proportional to the concentration from 0.2 – to 8 ng mL^{-1} of Anti-HCV (square R equal to 0.993 and relative

error <1%). When analyzing Fig. 6, we observed that in up to 10 ng $mL^{-1}$of Anti-HCV, the curve calibration reached the maximum level of interaction, with the immunocomplex formed. The limit of detection (LOD = 3.3 x standard deviations of y (σ) / slope of the calibration curve (S)) was calculated according to the linear regression equation of the calibration curve (ΔI = 0,89x + 0,1173 [Anti-HCV]), by processing data in Origin Pro SRO v8.0724. An LOD = 0.07 ng mL^{-1} Anti-HCV.

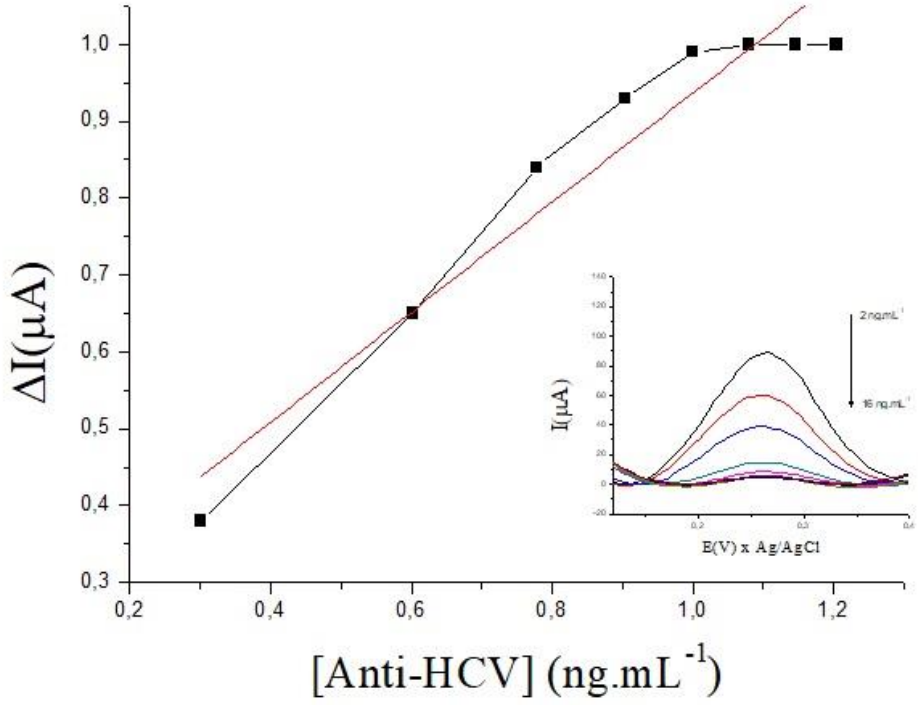

Fig. 6. Analytical curve of the PTh-GO-CTAB nanoelectrode for different Anti-HCV concentrations (2-16 ng mL^{-1}) obtained by SWV measurements in K3Fe(CN)6/ K4Fe(CN)6 (0.005molL_1) prepared in KCl (0.1molL_1) (Inset: SWV of the 2-16 ng mL^{-1}).

3.3 Response to Anti-HCV in real samples

For a more accurate response of the immunosensor, it was chosen to use a more sensitive technique than the CV, being used the SWV. The sensor was subjected to serum samples. Fig. 7 B given the SWV profile of successive incubation responses to the 5 ng / mL anti-HCV samples. The immunosensor showed responsiveness in samples up to 45 ng / mL^{-1}. These findings were confirmed by linearizing the curve of Fig.7 A to scale, indicating that the responses remained linear with increasing antibody concentration between 5 and 45 ng / mL^{-1} anti-HCV and with a limit of detection of approximately 3.3 ng / mL (n = 6, p <0.01) similar to Moraes et al., (Moraes et al., 2013)

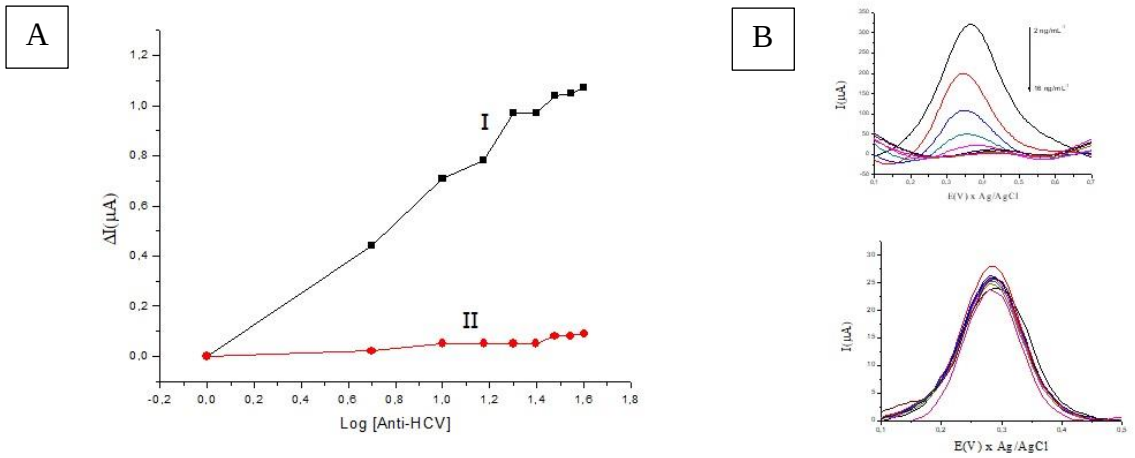

Fig. 7 A) Analytical curve of response to antibodies present in positive (curve I) and negative (curve II) anti-HCV under optimal experimental conditions; B) successful deposits of anti-hcv; successful deposits of serum.

4. Conclusions

In this study, we fabricated a sensitive immunosensor to detect Anti-HCV with in blood samples. In this design, a nanocomposite was synthesized electrochemically using the interaction between PTh and CTAB-mediated GO leaves in a single step. Apart from this technique, SWV was utilized to monitor electrode modification steps and was utilized to prove HCVcAg-Anti-HCV interaction. The bioconjugation of the nanocomposite to the streptavidin in the sensor surface was proven through the electrochemical findings, allowing the stable immobilization of several biotinylated antigen biomolecules. The sensitivity of immobilization techniques coupled with the use of GO and the selectivity of the reactions

between antibody and antigen will result in more sensitive and reliable electrochemical immunoassays. The nanocomposite prepared in a one-step under nanostructured film is a potential for immunosensor development, to detect antibodies as well as circulating antigens in biological samples.

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References.

Bhardwaj, J., Sharma, A., Jang, J., 2019. Biosensors and Bioelectronics, 126, 36–43.

Chung, C., Kim, Y., Shin, D., Ryoo, S., Hong, B. H., Min, D., 2013. ' Accounts Of Chemical Research, 46, 2211–2224.

Dutra, R. F., Kubota, L. T., 2007. Clinica Chimica Acta, 376, 114–120.

Jefferies, M., Rauff, B., Rashid, H., Lam, T., Rafiq, S., 2018. World J Clin Cases, 6, 589-599.

Kawagishi, N., Suda, G, Onozawa, M, Kimura, M., Maehara, O., Ito, J., Nakai, M., Sho, T., Natsuizaka, M., Morikawa, K., Ogawa, K., Sakamoto, N., 2017. Journal of Hepatology, 67, 1106–1121

Khan, S. T., Karges, W., Cooper, C. L. Crawley, A. M., 2017. John Wiley & Sons Ltd, Immunology, 154, 156–165.

Kim, J., Park, S., Min, D., 2017. Anal. Chem., 89, 232−248.

Lima, L. R., Gonçalves, A. B., Paulovich, F. V., Oliveira Jr., O. N., Ribeiro, S. J. L., Moraes, M. L., 2018. J. Braz. Chem. Soc.,. 29, 2054-2059.

Mahesh, S., Tang, K., Raj, M., 2018. Molecules, 23, 1-43.

Mavilia M. G., Wu, G. Y., 2018. J Clin Transl Hepatol, 6, 296–305.

Moraes, M. L., Lima, L. R., R. Silva, R. R., Cavicchioli, M., Ribeiro, S. J. L., 2013. Langmuir, 29, 3829−3834

Nakano, T., Moriya, k., Koike, k, Horie, T., 2018. Plos One, 1, 1- 14.

Saeed, R., Usman, M., Rasool, N., Ahmad, M., Khan, Z. A., Farooqi, Z. H., Siddiq, M., Zahoor, A. F., 2017. Journal of Molecular Liquids, 240, 389–394.

Singhal, C., Ingle, A., Chakraborty, D., Krishna PN, A., Pundir, C. S., Narang, J., 2017. International Journal of Biological Macromolecules, 98, 84–93

Shamsayei, M., Yamini, Y., Asiabi, H., 2016. Journal of Chromatography A, 1475, 8–17.

Shawky, S. M., Awad, A. M., Allam, W., Alkordid, M. H., EL-Khamisy, S. F., 2017. Biosensors and Bioelectronics, 92, 349–356.

Silva, M. M. S; Dias, A. C. M. S.; Silva, B. V. M.; Gomes-Filho, S. L. R.; Kubota, L. T.; Goulart, M. O.; Dutra, R. A.F. 2014. **J Chem Technol Biotechnol,** v.14, 23-28.

Sun, L., Yu, J., Sh, Y., Zhang, X., Shu, M., Chen, M., 2018. J Med Virol., 90, 926–935.

Sun, H., Uemura, H., Wong, N., Chan, D. P., Wong, B. C., Lin, P., Su, L., Hung, C., Oka, S., Chang, S., Lee, S., 2018. Live International, 1, 1-25.

Trindade, E. K. G., Dutra, R. F., 2018. Colloids and Surfaces B: Biointerfaces, 172, 272–279.

Uliana, C. V., Riccardi, C. S., Yamanaka, H., 2014, World J Gastroenterol, 20, 15476-15491.

Waner, M. J. Hiznay, J. M., Mustovich, A. T., Patton, W. M., Ponyik, C., Mascotti, D. P., 2019. Biochemistry and Biophysics Reports, 17, 127–131.

Wang, C., Jiang, T., Zhao, K., Deng, A., Li, J., 2019. Talanta, 193, 184–191.

Wang, L., Zhang, Y., Wu, A., Wei, G., 2017. Analytica Chimica Acta, 985,24-40.

Watanabe, T., Hashida, S., 2018. Journal of Immunological Methods, 459, 76–80.

Valva, P., Ríos, D. A., Matteo, E. D., Preciado, M. V., 2016. World J Gastroenterol, 22, 1367–1381.

5.3 ARTIGO 3 - A label-free electrochemical genosensor for Hepatitis C based on Poly-L-Lysine/carbon nanotube hybrid film.

Artigo à ser submetido no periódico Analytica Chimica Acta

Impact factor: 4.950

A label-free electrochemical Genosensor for Hepatitis C based on Poly-L-Lysine/carbon nanotube hybrid film.

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ABSTRACT

Hepatitis C virus (HCV) affects about 3% of the world population. Nearly 80% of infected individuals develop chronic disease, often associated with liver cirrhosis and hepatocellular carcinoma. Polymer surface-based biosensor using carbon nanomaterials have been widely applied to electrochemical biosensors. A poly-L-lysine (PLL) is a positively charged synthetic polymer of the amino acid(s) l-lysine or d-lysine. PLL films facilitates the attachment of proteins, DNA and cells. Herein, carboxylated multi-walled carbon nanotubes (COOH-CNT) and PLL were linked to form a 3D carbon nanostructure, with a high electron transfer rate due to vertically aligned CNT. The construction stages of the sensor platform and analytical sensor response were analyzed by electrochemical techniques of cyclic voltammetry (CV) and square wave voltammetry (SWV). The addition of CNT promoted increase in the sensing area and improved stability of the surface. With the proposed methodology was possible to develop a nanostructured film for determination of HCV at clinical importance levels. The proposed genosensor based on the COOH-CNT and PLL showed to be sensitive and specific to target complementary oligonucleotides containing the conserved sequence of HCV. The electrochemical response was registered after incubation with complementary DNA, confirming the hybridization by decrease of amperometric response obtained by SWV. The sensor was responsive to HCV spiked serum samples.

Keywords: biosensor, point-of-care, nanomaterial, biopolymer, Hepatitis, DNA.

1. Introduction

The Hepatitis C virus (HCV) is a bloodborne human viral pathogen belonging to the *Flaviviridae* family with a single-stranded genome [1]. Because the primary mode of transmission occurs parenterally (vertical and sexual in some cases), in addition to the disease being asymptomatic, screening methods that may assist in containing the spread of HCV are desirable. The conventionally used diagnostic of HCV infection is performed in two steps, first is screening for Anti-HCV antibodies with a serological test that identifies people who have been infected with the virus. If the test is positive for Anti-HCV antibodies, a nucleic acid test for HCV ribonucleic acid (RNA) is needed to confirm, determine the stage of infection, and identify the viral genotype, making possible the choice of appropriate treatment [2]. These tests require processing in a laboratory unit, sometimes presenting high cost and low levels of specificity and selectivity [3]. A good alternative for the rapid and sensitive diagnosis of HCV is the electrochemical based-DNA sensor, denominated genosensor [6].

Electrochemical detection of DNA probes, through their hybridization, can be performed indirectly (using probes and labels) or directly (label-free) [3]. Some DNA detection genosensors have been developed based on the hybridization of short or hairpin sequences using different detection techniques such as cyclic and square wave voltammetry [5]. These techniques are the most suitable for characterization of sensor platforms, because they allow to follow the stages of electrode surface modification as a function of electrical parameters, such as current density and capacitance.

One of the important factors in the development of electrochemical genosensors is obtaining conductive surfaces with attractive properties, such as good electrical conductivity, high surface area, biocompatibility, chemical and electrochemical stability [6]. Carbon nanotubes are extremely suitable for the development of these sensors because they have good electrical properties and can be functionalized with reactive groups suitable for binding of biological recognition molecules, especially the carboxylated Carbon Nanotubes (COOH-CNT) [7]. The carboxylation of CNTs is composed by the process of acidification or thermal synthesis, with several methodologies described [8]. Although the carboxylation compromises the electrical conductivity, it provides a great advantage, including the possibility of anchoring biomolecules by the amino terminal portion, through a covalent and stable amide bond. Some nucleic acid sequence binding strategies include the modification of sequences by

the introduction of amine groups (amine ss-DNA) into the terminal portion, for the construction of genosensors [9].

Although great advances in sensitivity have been achieved by the introduction of CNT-COOH in electrochemical sensors, their unique use on the sensing surface does not guarantee a good reproducibility and can be leached during measurements due to their poor surface binding and minimize the transfer of electrons because they are randomly dispersed [10]. To minimize this inconvenience, binding agents such as polymers can be used in bioanalytical applications due to their inherent charge transport properties and biocompatibility, in biosensor applications showing advantages owing to their specific sensitivity to very minor perturbations [11].

PLL is a linear cationic polymer rich in amine groups, which allows its conjugation both with nanomaterials having carboxyl groups (-COOH) or epoxies, (-O-), and with biomolecules. The PLL has been studied because it presents characteristics of interest, such as being biocompatible, biodegradable, without toxicity and antigenicity, being possible to dissolve it in water [12,13,14]. The electrodeposition of PLL on the sensing surface is the most adequate means of immobilization because it allows a stable synthesis and control of film thickness formed in the sensing surface [15].

Herein, carboxylated multi-walled carbon nanotubes (COOH-CNT) and PLL were linked to form a 3D carbon nanostructure, with a high electron transfer rate due to vertically aligned CNT. Therefore, the COOH-CNT/PLL assembly with plentiful amino groups will open the way to a large number of opportunities, such as bioactive molecular attachment and the preparation of nanocomposites [16] In this study, an electrochemical genosensor was developed based on Poly-L-lysine and Carbon Nanotubes for the detection of the Hepatitis C virus.

2. Material and methods

2.1 Reagents

PLL were obtained from Sigma Aldrich (St.Louis, USA). Multi-walled carbon nanotubes functionalized with carboxylic groups (COOH- CNTs) (average diameter of ~ 10 nm, average length of 1–2 mm and 95% purity) were acquired from DropSens (Oviedo, ESP). Glycine, dimethylformamide (DMF), potassium ferrocyanide ($K_4Fe(CN)_6$) and potassium ferricyanide ($K_3Fe(CN)_6$) were purchased from Vetek (SãoPaulo, BRA). N-hydroxysuccinimide (NHS), N-ethyl-N′-(3-dimethylami-nopropyl) carbodiimide (EDC) were acquired from Sigma-Aldrich (St.Louis, MO, USA). The ultrapure water obtained from a water purification system Milli-Q (Billerica, USA) (18MΩ) was utilized to prepare all solutions.

All synthetic oligonucleotides were purchased from DNA Express Biotecnologia Ltda (São Paulo, Brazil), with the following sequences:

- Probe DNA (HCV-NS5B- amino-F): 5'(AminoC6)gggtaccgcccttgcgagtc3'
- Complementary DNA (HCV-NS5B- R): gactcgcaagggcggtaccc
- Noncomplementary DNA (HCV-NS5B-F**):** 5'gggtaccgcccttgcgagtc3'

2.2 Apparatus

The electrochemical studies were performed by using a portable potentiostat/galvanostat Ivium Compact Stat (Eindhoven, NLD) connected to a microcomputer and controlled by Ivium Soft software. It was used a three-electrode system, comprising a glassy carbon electrode (GCE) (Ø = 3 mm) as the working electrode, a helical platinum wire as counter electrode and Ag/AgCl (KCl saturated) as the reference electrode. Electrochemical analyses were carried out using an electrochemical cell (10 mL) and conducted at room temperature (approximately 24 °C).

The structural characterization was accomplished by Fourier Transform Infrared in the Attenuated Total Reflectance mode (ATR FT-IR) using the Bruker IFS 66 model FT-IR spectrometer (Billerica, USA). Spectra were acquired at 4000 cm^{-1} - 500 cm^{-1}. Scanning electron microscopy (SEM) assays were performed at the Keizo Asami Immunopathology

Laboratory (LIKA). A Scanning Electron Microscope JEOL - JSM 5600LV of the Technological Integration Center-1/Aggeu Magalhães Research Center (CPqAM) – FIOCRUZ was used, through a glassy carbon disc (~ 0.5 cm diameter) that was adapted to the electrochemical cell for further modifications.

2.3 Preparation of the glassy carbon electrode surface

Prior to modification (Fig. 1 A), the GCE was polished with alumina powder (1 and 0.5 mm) for 5 minutes until obtaining an impurities free surface. In order to remove any impurities, cleaned electrode was carefully rinsed with ultrapure water. After this step, the PLL (0.05 mmol. L^{-1}) prepared in PBS solution (0.01 mol. L^{-1}, pH 7.4) was electrochemically deposited on the GCE surface through the Cyclic voltammetry (CV) technique in the potential range - 2.0 to 2.0 V at scan rate of 0.05 Vs^{-1} for 15 cycles. Then, three layers of the COOH-CNT solution (15 mL) was deposited onto the GCE surface modified with PLL. The COOH-CNT solution consisted of 1mg of COOH-CNT dispersed in 1 mL of DMF and sonicated in an ultrasonic bath for 2 h. Each layer was dried at 40 °C for evaporation of the solvent.

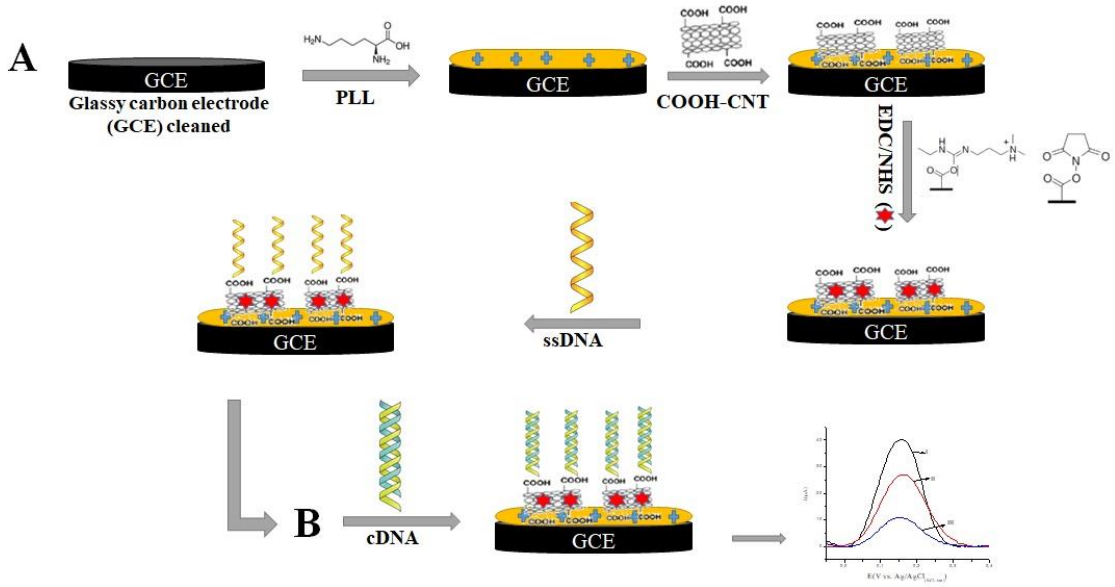

Fig. 1. Schematic representation of the (A) imunossensor fabrication and (B) electrochemical principle of detection

2.4 Immobilization and hybridization of Synthetic oligonucleotides on the nanoelectrode

Synthetic oligonucleotides (ssDNA) (300 pmol) were immobilized on the nanoelectrode during 40 minutes. The working electrode was maintained in a moist chamber at room temperature (~ 25 °C). The analytical performance of the as prepared nanoelectrode was evaluated through the incubation with 5µL of target oligonucleotide complementary (cDNA) in the concentration of 100 pmol, during 40 minutes (~55 °C). Analytical responses of cDNA interactions at the interface of the ssDNA-GCE were monitored by square wave voltammetry (SWV). SWV measurements were recorded between 0.0 and 0.5 V at 10 Hz with a pulse amplitude of 10 mV and a step potential of 0.0025 V. The stepwise modifications of the biosensor were accomplished through CV measurements. The analysis was carried out in a potential range of -0.2 to 0.6 mV, at 0.05 V s^{-1} scan rate, in presence of 0.005 mol L^{-1} of the $K_3Fe(CN)_6/K_4Fe(CN)_6$ prepared in KCl solution (0.1 mol L^{-1}).

3. Results and discussion

3.1 Electrochemical characterization of PLL-CNT film in GCE.

FTIR analyzes were performed to characterize the interaction of PLL with COOH-CNT and the formation of nanostructured film on the sensing interface. Fig. 2. shows the spectra of COOH-CNT (Curve I), PLL (Curve II), and PLL/ COOH-CNT (Curve III). The FTIR spectrum of the COOH-CNT shows typical peaks of carboxylic groups at 3350 cm^{-1}, which corresponds to the presence of OH groups, and another peak at 1734 cm-1 resulting from molecular elongation of C=O group [10]. The PLL spectrum showed several bands at 3325 cm^{-1} resulting from symmetric and asymmetric vibration of primary amines groups ($-NH_2$) of the polymer, and other bands at 1623 – 1093 cm^{-1}, associated with the groups amide I (C=O) and amide II (C-N) [17]. In the PLL spectrum after binding of COOH-CNT appears a unique and elongated peak in 3371 cm^{-1} and another peak at 1578 and 1414 cm^{-1}, confirming that the nanotubes were bound to the polymer.

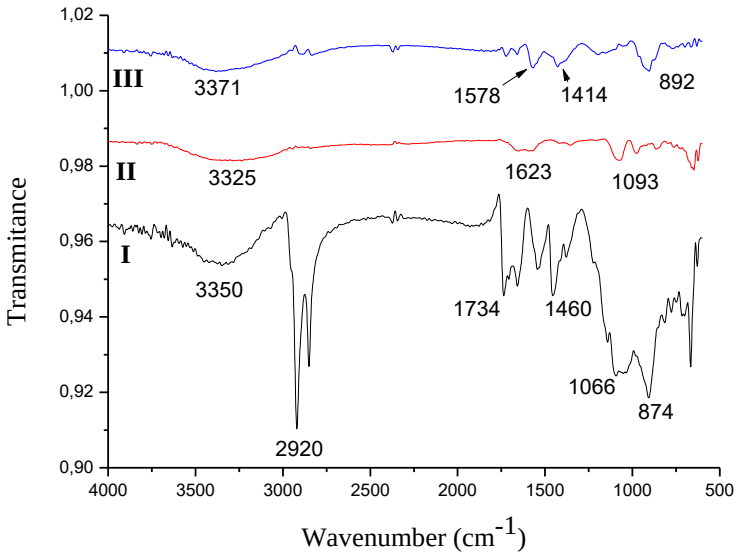

Fig. 2. FTIR spectra of the GCE modified with (I) COOH-CNT, (II) PLL and (III) COOH-CNT/PLL.

The stepwise modification of GCE surface was also characterized by SEM technique. Firstly, Fig. 3A shows GCE surface after electrodeposition of the PLL film with a thin layer of the polymer, it is observed the formation of small polymer agglomerates on the surface of the electrode. The micrograph of Fig. 3B exhibited an irregular surface formed by abundant spaghetti-like filamentous structures attributed to the COOH-CNT presence [18]. The deposition of carbon nanotubes on PLL/GCE surface showed a porous three-dimensional nanostructured film [19].

The electrochemical profile of the platform nanostructured by PLL-NTC was investigated initially using CVs technique in $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ as redox probe. Fig. 3 C and Dshows the bare electrode (curve I), modified with PLL (curve II) and modified with PLL-CNT (curve III). Analysis of redox peaks of voltammograms after modification of GCE with PLL (0.05 mmol L^{-1}) exhibits a reduction in the current amplitude when compared to the cleaned electrode, this result is attributed to the non-conducting nature of the polymer [19]. After deposition of COOH–CNT on the PLL film, the voltammogram showed an increase in electroactive area that can be ascribed to higher electron transfer promoted by the increase of conductivity, due to the COOH-CNTs [14].

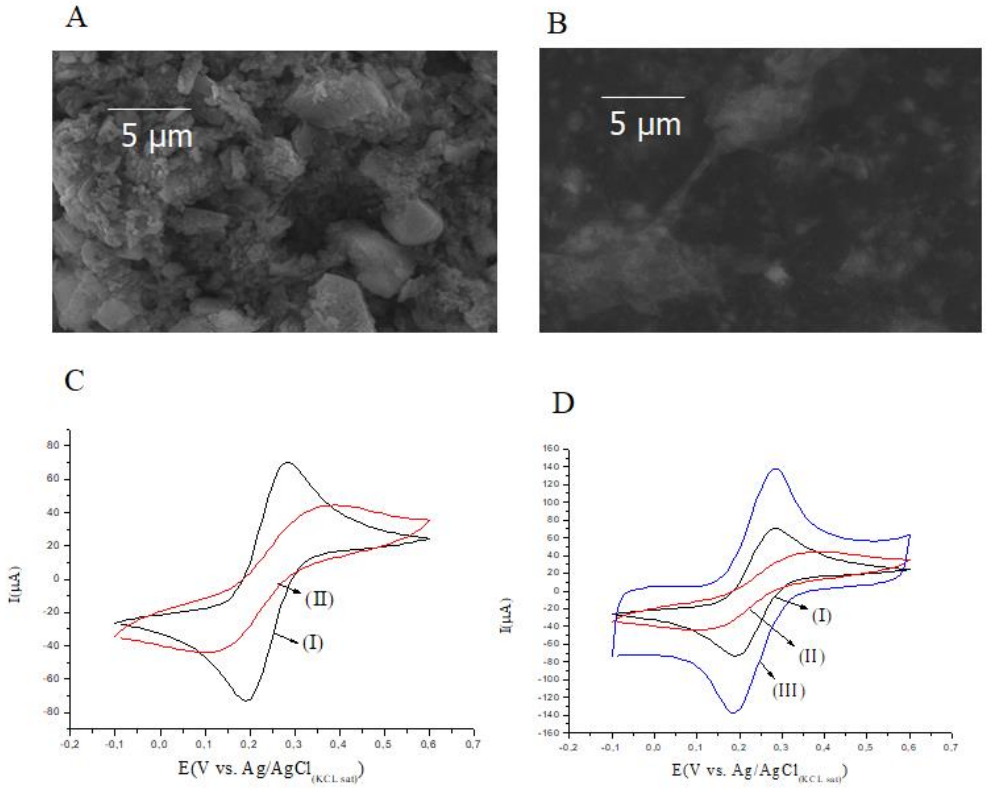

Fig. 3. SEM images and CVs of the surface (A) PLL/GCE and (B) CNT/PLL/GCE. In C) and D), (I) GCE after cleaning; (II) PLL/GCE; (III) COOH CNT/PLL/GCE. Measurements performed in K3Fe $(CN)_6$ /$K_4Fe(CN)_6$ (0.005 mol L^{-1}) prepared in KCl solution (0.1mol L^{-1}).

The electron diffusion study of the COOH-CNT-PLL/GCE was investigated submitting the electrode to different scan rates. As can be seen in Fig. 4, the voltammograms registered in a $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ (0.005mol L^{-1}), exhibiting a proportional increase in both cathodic and anodic peak currents (Ipa and Ipc, respectively) with increase of the square root of scan rate (10 to 150 mVs^{-1}). Ipa and Ipc were directly proportionals to the square root of scan rates (Fig. 2 - inset) with the following linear regression equations: Ipa (µA) 32,626x – 81,112 (r = 0.9902) and Ipc (µA) y = -30,307x + 64,187 (r = 0.9956). These results suggest that the reactions on the sensor interface were controlled by mass transport (surface diffusion) [20].

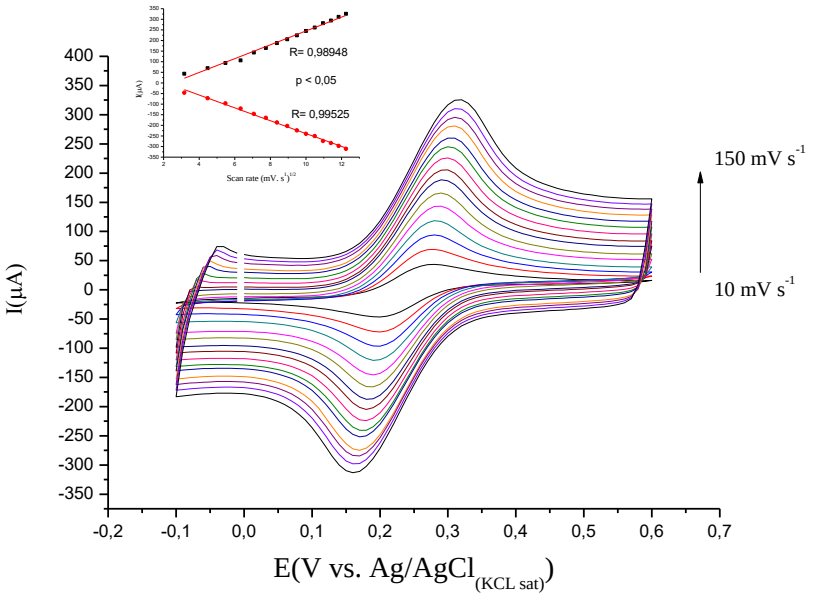

Fig 4. Voltammetric profile of the COOH-CNT-PLL/GCE under different scan rates (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 mVs^{-1}) (Inset: plots of the Ipa and Ipc vs. square roots of the scan rates). All the measurements were performed in $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ (0.005 mol L^{-1}) prepared in KCl solution (0.1 mol L^{-1}).

The film stability was evaluated by accomplishing the COOH-CNT-PLL\GCE to 20 voltammetric cycles, in a potential window varying from -0.2 to 0.6 V at 50mVs^{-1} of scan rate. According to Fig. 5, the redox peaks were maintained practically constant during all scannings. The coefficient of variation were 0.45 % and 0,32 % for anodic and cathodic peaks, respectively, indicating that the COOH-CNT-PLL\GCE film presented a high stability. This could be attributed to the PLL that stabilized the CNT and avoiding bundles in response to strong covalent interactions [21]. Presumable attachment formed between CNTs and PLL allowed a immobilization matrix with great stability.

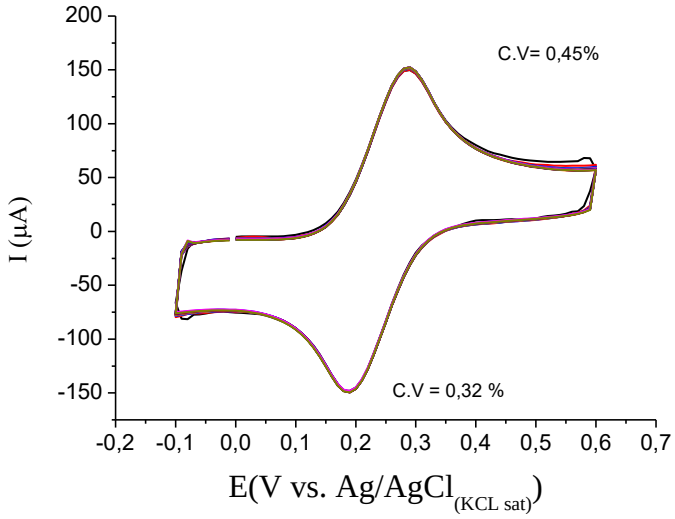

Fig 5. Successive cyclic voltammetry of the COOH-CNT-PLL/GCE.

3.2 Experimental optimizations

In order to achieve the optimal performance of the COOH-CNT- PLL film, it was varied the COOH-CNTs concentrations deposited on the PLL film (0.5 mg mL^{-1} to 3.5 mg mL^{-1}). The current peaks increased up to 1.5 mg mL^{-1} CNT as indicating by a plateau (Fig 6 A).

It was also investigated the influence of DNA probe concentrations on the sensor platform (Fig 6 B). In this study, a proportional increase in the amplitude of the peak currents in relation to ssDNA concentrations was found until reach a maximal response at 300 pmmol L^{-1}.

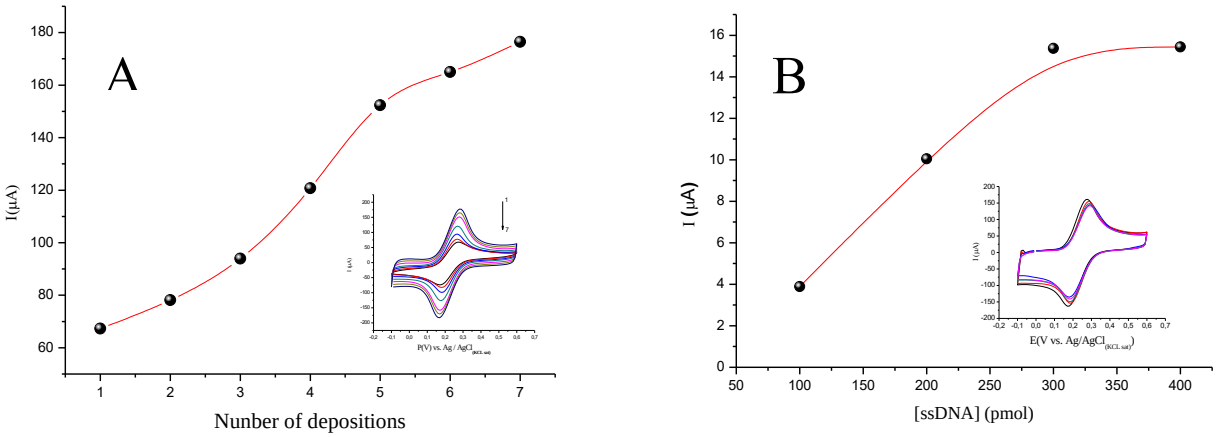

Fig 6. A) Plot of the Ipa vs. number of depositions of CNT (Inset: Cyclic voltammetry of the successive depositions of CNT). B) Ploto of the Ipa vs. nunber of depositions of ssDNA (Inset: Cyclic voltammetry of the successive depositions of ssDNA).

3.3 Analytical response

SWV electrochemical technique was employed to monitor complementary hybridizations of ssDNA on the electrode surface. In Fig. 6a, it is possible to observe SWV curve after immobilization of the ssDNA (curve II) with a decrease of current peak in relation to the profile presented by the nanocomposite film COOH-CNT / PLL sensor platform (curve I). Believed to occur due to the immobilization of the probe DNA on the sensor platform, such an outcome can be explained since the amino groups of poly-l-lysine can form an electrostatic affinity with the phosphate skeleton of DNA molecule to immobilize the probe DNA [6]. In curve III it is possible to observe a decrease in the current peak from the deposition of the complementary DNA probe (cDNA), suggesting that the hybridization has occurred. In 6 B it is possible to observe the negative controls of the tests (curve III), demonstrating the effectiveness of the test. The assays were also performed in serum (6 C and 6 D), in which results similar to those obtained in buffered medium were achieved.

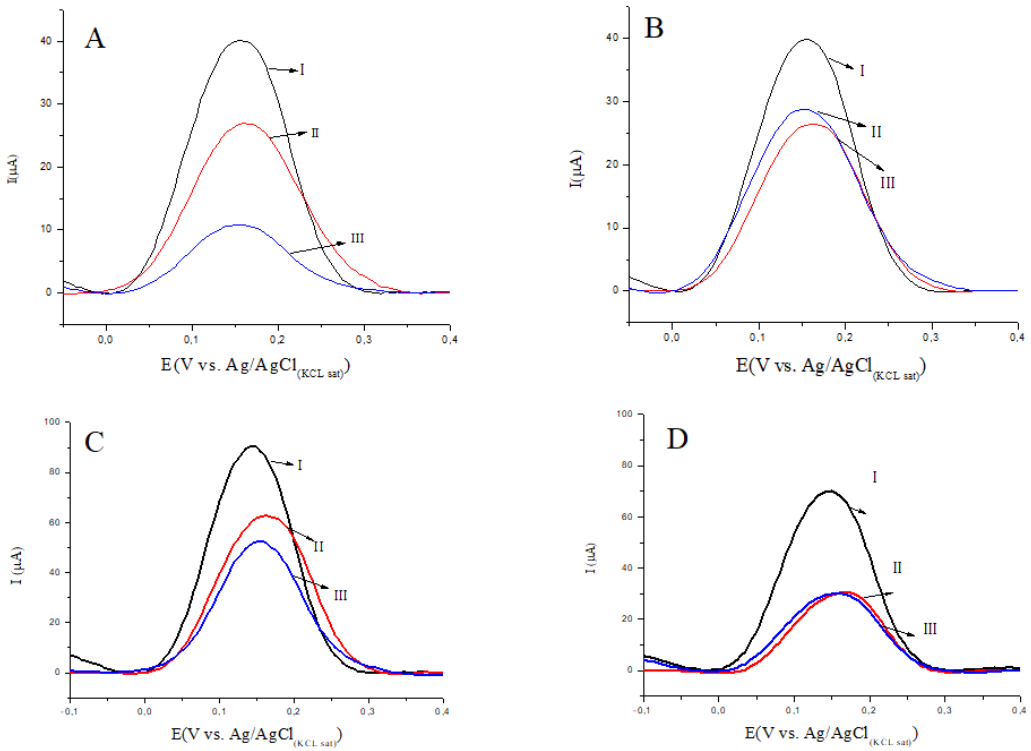

Fig 7. Square-wave voltammograms demonstrating the construction of the sensor platform, A) (I) COOH-CNT/PLL/ECV; (II) ssDNA (III) cDNA . B) (I) COOH-CNT/PLL/ECV (II) ssDNA (III) ncDNA. c) (I) COOH-CNT/PLL/ECV; (II) ssDNA (III) cDNA (in serum). d) (I) COOH-CNT/PLL/ECV (II) ssDNA (III) serum All the measurements were performed in $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ (0.005 mol L^{-1}) prepared in KCl solution (0.1 mol L^{-1}).

4. Conclusions

The proposed genosensor based on the COOH-CNT and PLL showed to be sensitive and specific to target complementary oligonucleotides containing the conserved sequence of HCV. The electrochemical response was registered after incubation with complementary DNA, confirming the hybridization by decreased of amperometric response obtained by SWV The sensor was responsive to HCV spiked serum samples.

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References

[1] D. C. Nyan, K. L. Swinson, International Journal of Infectious Diseases 43(2016) 30–36.

[2] S. M. Shawky, A. A. Awad, W. Allam, M. H. Alkordi, S. F. El-Khamisy, Biosensors and Bioelectronics 92 (2017) 349-356.

[3] C. Singhala, C.S. Pundirb, J. Naranga, Biosensors and Bioelectronics 97 (2017) 75–82.

[4] Q. Jiang, Y. J. Chandar, S. Cao, E. D. Kharasch, S. Singamaneni, J. J. Morrissey, Adv. Biosys. DOI: 10.1002/adbi.201700096. 2017.

[5] M.S. Hejazi, M.H. Pournaghi-Azar, F. Ahour, Analytical Biochemistry 399 (2010) 118–124

[6] G. Liang, Y. Man, A. Li, X. Jin, X. Liu, L. Pan, Microchemical Journal 131 (2017) 145–153

[7] Z. Zhu, L. Garcia-Gancedo, A. J. Flewitt, H. Xie, F. Moussy, W. I Milne, Sensors. *D*oi:10.3390/s120505996, 2012.

[8] A. Dasgupta, L. P. Rajukumar, C. Rotella, Y. Lei, M. Terronesa, Nano Today 12 (2017) 116–135

[9] S. M. Majd, A. Salimi, Analytica Chimica Acta 1000 (2018) 273 e 282

[10] P. M. S. Silva, A. L. R. Lima, B. V. M. Silva, L. C. B. B. Coelho, R. F. Dutra , M. T. S. Correia, Biosensors and Bioelectronics 85(2016)171–177.

[11] M. M. Barsan, M. E. Ghica, C. M.A. Brett, Analytica Chimica Acta 881 (2015) 1–23

[12] N. Sahiner, Polymer 121 (2017) 46-54.

[13] M. A. D. Millone, , E. A.Ramirez, C. Y. Chain, A. Crivaro, D. Romanin, M. Rumbo, G. Docena, M. D. Cocco, M. L. Pedano, A. Fainstein, J. Montoya, M. E. Vela, R. C. Salvarezza, Journal of Nanomaterials. http://dx.doi.org/10.1155/2016/5432656, 2016.

[14] B. Thirumalraj, S. Kubendhiran, S. M. Chen, K. Y. Lin, Journal of Colloid and Interface Science 498 (2017) 144–152.

[15] Y. Zhao, Y. Zhang, Z. Zhuge, Y. Tang, J. Tao, Y. Chen, Analytical Chemistry, 90 (2018) 3149−3155

[16] D. Wasik, A. Mulchandani, M. V. Yates, Biosensors and Bioelectronics 91 (2017) 811–816.

[17] Y. Jalit, M. C. Rodriguez, M. D. Rubianes, S. Bollo, G. A. Rivasa, Electroanalytical 20 (2008), 1623-1631.

[18] C. Jiang, T. Yang, K. Jiao, H. Gao, Electrochimica Acta 53 (2008) 2917–2924

[19] Y. Xue, L. Bao, X. Xiao, L. Ding, J. Lei, H. Ju, Analytical Biochemistry 410 (2011) 92–97

[19] P. M. S. Silva, A. L. R. Lima, B. V. M. Silva, L. C. B. B. Coelho, R. F. Dutra , M. T. S. Correia, Biosensors and Bioelectronics 85(2016)171–177.

[20] X. Kang, J. Wang, H.Wu, I. A. Aksay, J. Liu, Y. Lin, Biosens. Bioelectron. 25 (2009) 901–905.

[21] Y. Zhang, J. Li, Y. Shen, M. Wang, J. Li, J. Phys. Chem. B 108 (2004), 15343-15346

[22] D. G. A. Cabral, E C. S. Lima, P. Moura, R. F. Dutra, Talanta 148 (2016) 209–215

5 CONCLUSÕES

A utilização nanomateriais de carbono associados a polímeros amino/carbóxi possibilitou a construção de sensores eletroquimicamente estáveis e reativo-funcional resultando em maior reprodutibilidade dos filmes nanoestruturados, maior transferência eletrônica e área superfície/volume;

Foram desenvolvidas e caracterizadas três plataformas para a detecção do anticorpo e uma sequência de RNA do vírus da Hepatite c. Nos dois primeiros estudos, constata-se que o emprego de nanomateriais em conjunção à monômeros possibilita eletropolimerização *in situ* de modo mais simples e com única etapa, de modo mais controlado. No último estudo utilizou-se a técnica de deposição camada por camada. As técnicas de CV e SWV foram eficientes na análise das etapas de modificação do eletrodo e no estudo analítico para detecção das biomoléculas;

Foi desenvolvido um imunossensor utilizando eletrodo de carbono vítreo com Polipirrol e Óxido de Grafeno (PPy@GO), gerando um efeito sinérgico que resultou em um aumento da condutividade elétrica em cerca da 1.8 vezes em comparação com filme de polipirrol, no qual foi possível a imobilização o antígeno-HVC para a detecção do Anti-HCV;

A modificação do eletrodo de ouro com o filme nanoestruturado de Politiofeno e Óxido de Grafeno (PTh-GO), sendo possível a imobilização o antígeno-HVC para a detecção do Anti-HCV;

A bioconjugação dos nanocompósitos de PPy@GO e PTh-GO à estreptavidina na superfície sensora foi comprovada através dos achados eletroquímicos, permitindo então a imobilização estável de diversas biomoléculas biotiniladas;

A associação de NTC-COOH ao filme de PLL proporcionou um aumento na área eletroativa, melhora da condutividade elétrica promovida pelos nanotubos de carbono e aumento da estabilidade da superfície;

É possível concluir que os protótipos desenvolvidos permitiram a detecção das biomoléculas utilizadas em níveis de interesse clínico para uso em amostras sanguíneas, sem necessidade de diluição ou pré-concentração e possibilitam o desenvolvimento de testes rápidos de baixo custo e de alta reprodutibilidade, comparado aos sistemas ópticos (ressonância de plásmons de superfície) e piezoelétricos. Dado a grande versatilidade da tecnologia, novas aplicações podem ser desenvolvidas com a substituição da molécula

sensora, assim diferentes aplicações de biossensores poderão ser desenvolvidas com esta mesma nova tecnologia proposta, podendo ser empregados na triagem de doadores sanguíneos, auxiliando a facilitando a logística da coleta.

REFERENCIAS

ABBAS, A. K; LICHTMAN, A. H.; PILLAI, S. **Imunologia celular e molecular**. 7ª ed. Rio de Janeiro: Elsevier, 2012.

ABDEL-HAMEED, E. A.; ROUSTER, S. D.; ZHANG, X.; CHEN, J.; MEDVEDOVIC, M.; GOODMAN, Z. D.; SHERMAN, K. E. Characterization of HCV NS3 Protease Variants in HCV/HIV-Coinfected Patients by Ultra-Deep Sequence Analysis: Relationship with Hepatic Fibrosis. **J Acquir Immune Defic Syndr**, v. 74, p.1-8, Mar,2017. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5303138/. Acesso em: 12 Jan. 2019.

ABREU, A.C. C.; SIPAÚBA, B. G.; ARAÚJO, C. M. D.; ARAÚJO, T. M. E. Perfil clínico-epidemiológico dos casos de hepatite B e C do Piauí. **R. Interd**. v.6, p.102-111, Ago 2013. Disponível em: https://revistainterdisciplinar.uninovafapi.edu.br/index.php/revinter/article/ view/214/0. Acesso em: 17 Abr. 2019.

AFZAL, A.; ABUILAIWI, F. A.; HABIB, A.; AWAIS, M.; WAJE, S. B; ATIEH, M. A. Polypyrrole/carbon nanotube supercapacitors: Technological advances and challenges. **Journal of Power Sources**. v. 352, p. 174-186, 2017. Disponível em: https://revistainterdisciplinar.uninovafapi.edu.br/index.php/revinter/article/ view/214/0. Acesso em: 17 Jan. 2019.

AHOUR, F., POURNAGHI-AZAR, M. H., ALIPOUR, E., HEJAZI, M. S. Detection and discrimination of recombinant plasmid encoding hepatitis C virus core/E1 gene based on PNA and double-stranded DNA hybridization. **Biosensors and Bioelectronics**, v. 45, p. 287–291, 2013. Disponível em: https://revistainterdisciplinar.uninovafapi.edu.br/index.php/revinter/ article/ view/214/0. Acesso em: 17 Mar. 2019.

ALVES, M. R.; SILVA, D. M.; SOUZA, T. O.; OLIVEIRA, Y. N. S.; NERY, A. A.; CASOTTI, C. A. Perfil epidemiológico dos casos de hepatite C em uma diretoria regional de saúde da Bahia. **J. res.: fundam. Care**. v. 6, p. 889-896, 2014. Disponível em: https://revistainterdisciplinar.uninovafapi.edu.br/index.php/revinter/article/ view/214/0. Acesso em: 18 Jan. 2019.

ARONOFF-SPENCER, E., VENKATESH, A. G., HOWARDBRICKNER, DAVIDLOONEY, DREW . HALL. Detection of Hepatitis C core antibody bydual-affinity yeast chimera and smartphone-based electrochemical sensing. **Biosensors and Bioelectronics**, n. 86, p. 690–696, 2016. Disponível em: https://www.sciencedirect.com/science/article/pii/S0956566316306510?via%3Dihub Acesso em: 18 Jan. 2019.

AYDEMIR, N.; MALMSTROM, J.; TRAVAS-SEJDIC, J.;Conducting polymer based electrochemical biosensors. **Physics and Chemistry**. v. 18, p. 8264-8277, 2016. Disponível em: https://pubs.rsc.org/en/content/articlelanding/2016/cp/c5cp06830d#!divAbstract. Acesso em: 15 Jan. 2019.

BARSAN, M. M. GHICA, M. E., BRETT, C.M.A. Electrochemical sensors and biosensors based on redox polymer/ carbon nanotube modified electrodes: A review. **Analytica Chimica Acta**, n. 881, p.1–23, 2015. Disponível em: https://www.sciencedirect.com/science/article/pii/S0956566316306510?via%3Dihub Acesso em: 11 Jan. 2019.

BELYHUN, Y.; MAIER, M.; MULU, A.; DIRO, E.; GERD LIEBERT, U.; Hepatitis viruses in Ethiopia: a systematic review and meta-analysis. **BMC Infectious Diseases**. v. 16, p.761-769, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5168848/. Acesso em: 11 Abr. 2019.

BERTÓK, T.; KATRLÍK, J.; GEMEINER, P.; TKAC, J. Electrochemical lectin based biosensors as a label free tool in glycomics. **Microchemical Acta**, v. 180, p. 1-13, 2013. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/27239071/. Acesso em: 17 Abr. 2019.

BOSAN, A.; QURESHI, H.; BILE, K. M.; AHMAD, I.; HAFIZ, R. A review of hepatitis viral infections in Pakistan. **J Pak Med Assoc**. v. 60, p. 1045- 1058, 2010. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/27239071/. Acesso em: 17 Fev. 2019.

BRASIL. Ministério da Saúde. Secretaria de Ciência, Tecnologia e Insumos Estratégicos. Comissão Nacional de incorporação de tecnologias no SUS. PROTOCOLO CLÍNICO E DIRETRIZES TERAPÊUTICAS PARA HEPATITE C E COINFECÇÕES. BRASÍLIA, 2017. 111 p.

CANDIAN, L.M. **Estudo do Polietileno de alta densidade reciclado para uso em elementos estruturais.** 2007. Dissertação (Mestrado em Engenharia de Estruturas). Escola de Engenharia de São Carlos, Universidade de São Paulo, São Carlos, 2017.

CAMPOS-FERREIRA, D. S., SOUZA, E. V. M., NASCIMENTO, G. A., ZANFORLIN, D. M. L., ARRUDA, M. S., BELTRA, M. F. S., MELO, A. L., BRUNESKA, D. B., LIMA-FILHO, J. L. Electrochemical DNA biosensor for the detection of human papillomavirus E6 gene inserted in recombinant plasmid. **Arabian Journal of Chemistry**, v. 9, p. 443-450, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5168848/. Acesso em: 11 Abr. 2019.

CHASCSA, D. M., MOUSA, O. Y., PUNGPAPONG, S., ZHANG, N., CHERVENAK, A., NIDAMANURI, S., RODRIGUEZ, E., FRANCO, D., RYLAND, K., KEAVENY, A.P., HUSKEY, J. L., SMITH, M., REDDY, K. S., TANER, C. B., VARGAS, H. E., AQEL, B. A. Clinical outcomes of hepatitis C treatment before and after kidney transplantation and its impact on time to transplant: A multicenter study. ***Am J Transplant.*** v. 18, p. 2559–2565, 2018. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/27239071/. Acesso em: 30 Abr. 2019.

CHEVALIEZ, S., POITEAU, L., ROSA, I., SOULIER, A., ROUDOT-THORAVAL, F., LAPERCHE, S., HÉZODE, C., PAWLOTSKY, J. M. Prospective assessment of rapid diagnostic tests for the detection of antibodies to hepatitis C virus, a tool for improving access to care. **Clin Microbiol Infect,** v. 22, p. 459.e1–459.e6, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/27239071. Acesso em: 13 Abr. 2019.

DAI, H.; WANG, N.; WANG, D.; ZHANG, X.; MA, H.; LIN, M. Voltammetric uric acid sensor based on a glassy carbon electrode modified with a nanocomposite consisting of polytetraphenylporphyrin, polypyrrole, and graphene oxide. **Microchim Acta**. DOI 10.1007/s00604-016-1953-x. 2016. Disponível em: https://europepmc.org/article/med/31183562. Acesso em: 13 Abr. 2019.

DE MARTEL, C. World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma. **Hepatology**, v. 62, p. 1190-1200, 2015. Disponível em: https://europepmc.org/article/med/31183562. Acesso em: 17 Mar. 2018.

DIAS, A. C. M. S.; GOMES-FILHO, S. L. R.; MI´ZIA M.S.SILVA, M. M. S.; DUTRA, R. F. A sensor tipbasedoncarbonnanotube-inkprintedelectrode for the dengue virus NS1 protein. **Biosensors and Bioelectronics**. v. 44, p. 216–221, 2013. Disponível em: https://europepmc.org/article/med/31183562. Acesso em: 19 Jun. 2018.

DROSTE, H. M. D. **A PRÓXIMA GERAÇÃO DE DISPOSITIVOS DE DIAGNÓSTICO MOLECULARES IN-VITRO PORTÁTEIS PARA A DETEÇÃO DE MARCADORES BIOLÓGICOS.** 2017**.** Dissertação **(**Mestrado em Ciências Farmacêuticas), Instituto Superior de Ciências da Saúde Egas Moniz, São Paulo 2017.

DUNG, N. Q.; PATIL, D.; DUONG, T.; JUNG, H.; KIM, D.; YOON, S. Na amperometric glucose biosensor based on a GO x-entrapped TiO2 –SWCNT composite. **Sensors and Actuators B,** v.166– 167, p. 103– 109, 2012. Disponível em: https://www.academia.edu/11255371/An_amperometric_glucose_biosensor_based_on_a_GO x-entrapped_TiO2_SWCNT_composite Acesso em: 17 Jun. 2018.

DYAL, H. K.; AGUILAR, M.; BARTOS, G.; HOLT, E. W.; BHUKET, T.; LIU, B.; CHEUNG, R.; WONG. R. J. Diabetes Mellitus Increases Risk of Hepatocellular Carcinoma in Chronic Hepatitis C Virus Patients: A Systematic Review. **DigDisSci,** DOI 10.1007/s10620-015-3983-3. 2015. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26703125. Acesso em: 13 Jun. 2018.

EL-SERAG, HASHEM B. Risk of hepatocellular carcinoma after sustained virological response in Veterans with hepatitis C virus infection. **Hepatology**, v. 64, p. 130-137, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26946190. Acesso em: 17 Jun. 2018.

FILHO, A. G. S.; FAGAN, S. Funcionalização de Nanotubos de carbono. **Revista Química Nova**. v. 30, p. 1695-1703. 2007. Disponível em: http://www.scielo.br/scielo.php?pid=S0100-40422007000700037&script=sci_abstract&tlng=pt. Acesso em: 17 Jun. 2018.

FOCACCIA, R. Tratamento de Hepatites Virais e Doenças Associadas. 3ª Edição. São Paulo: Editora Atheneu, 2013. 957 p.

FOSTER, G. R., IRVING, W. L., CHEUNG, M. C. M., WALKER, A. J., HUDSON, B. E., VERMA, S., MCLAUCHLAN, J. M., MUTIMER, D. J., BROWN, A., GELSON, W. T. H., MACDONALD, D. C., AGARWAL, K., Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. **Journal of Hepatology**. v. 64. p. 1224–1231, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26829205. Acesso em: 18 Jun. 2019.

FREITAS, T. A.; MATTOS, A. B.; SILVA, B. V.; DUTRA, R. F. Amino-Functionalization of Carbon Nanotubes by Using a Factorial Design: Human Cardiac Troponin T Immunosensing Application. **BioMed Research International**. v. 14, p. 1- 9, 2014. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26829235. Acesso em: 18 Abr. 2018.

GAUDIN, V. Advances in biosensor development for the screening of antibiotic residues in food products of animal origin – A comprehensive review. **Biosensors and Bioelectronics**, v. 90, p. 363–377, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/268293654. Acesso em: 18 Abr. 2018.

GEDEDZHA, M. P., MPHAHLELE, M. J., BLACKARD, J. T., SELABE, S. G. Prevalence of NS5B Resistance Mutations in Hepatitis C Virus (HCV) Treatment Naive South Africans. **Hepat Mon**., v. 6, p. 14248 - 14255, 2017. Disponível em: http://hepatmon.com/articles/14248.html. Acesso em: 12 Ago. 2018.

GERRESHEIM, G. K., DU¨NNES, N., NIEDER-RO¨HRMANN, SHALAMOVA, L. A., FRICKE, M., HOFACKER, I., SIEDERDISSEN, C. N. Z., MARZ, M., NIEPMANN, M., microRNA-122 target sites in the hepatitis C virus RNA NS5B coding region and 30 untranslated region: function in replication and influence of RNA secondary structure. Cell. **Mol. Life Sci**. v. 74, p. 747–760, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/27677491. Acesso em: 19 Set. 2018.

GOMES-FILHO, S. L. R. et al. A carbon nanotube-based electrochemical immunosensor for cardiac troponin T. **Microchemical Journal,** v. 109, p. 10-15, 2013. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/27677491. Acesso em: 12 Ago. 2018.

GUEX, L. G.; SACCHI, B.; PEUVOT, K. F.; ANDERSSON, R. L.; POURRAHIMI, A. M.; V. STRÖM, V.; S. FARRIS, S.; OLSSON, R.T. Experimental review: Chemical reduction of graphene oxide (GO) to reduced graphene oxide (rGO) by aqueous chemistry.**Nanoscale.** v. 27, p. 9249 - 9772, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/27677465. Acesso em: 17 Ago. 2018.

GUO. X. Surface plasmon resonance based biosensor technique: A review. **J. Biophotonics** 5, n 7, p. 483–501, 2012. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/22467335. Acesso em: 19 Jan. 2018.

GUSTAFSSON, S. S.; EHRENBERG, A.; SCHMUCK, B.; ANWAR, M. I.; DANIELSON, U. H. Identification of Weak Points of Hepatitis C Virus NS3 Protease Inhibitors Using Surface Plasmon Resonance Biosensor-Based Interaction Kinetic Analysis and Genetic Variants. **J. Med. Chem.** v. 57, p. 1802−1811, 2014. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/22467378. Acesso em: 12 Abr. 2018.

GUZ B, STRAUSS E, TAKADA A, ARRUDA F, GAYOTTO LCC. Spontaneous disappearance of serum HCV-RNA in chronic hepatitis. **Hepatology.** v.30, p. 197 -203, 2009. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/22467335. Acesso em: 18 Fev. 2017.

HE, Z., ZANG, S., LIU, Y, HE, Y, LEI, H. A multi-walled carbon nanotubes-poly (L-lysine) modified enantio selective immunosensor for ofloxacin by using multi-enzyme-la- beled gold nanoflower as signal enhancer. **Biosensors and Bioelectronics**, v. 73, p. 85–92, 2015. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26047998. Acesso em: 12 Jan. 2019.

HEJAZI, M. S., POURNAGHI-AZAR, M. H., AHOUR, F, Electrochemical detection of short sequences of hepatitis C 3a virus using a peptide nucleic acid-assembled gold electrode. **Analytical Biochemistry**, v. 399, p. 118–124, 2010. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26047667. Acesso em: 13 Fev. 2019.

HUANG, Y.; FEILI, H.; WANG, Z.; ZHU, M.; PEI, Z.; XUE, Q.; HUANG, Y.; ZHI, C. Nanostructured Polypyrrole as a flexible electrode material of supercapacitor. **Nano Energy.** v.22, p. 422-438, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26047554. Acesso em: 11 Jan. 2019.

JIANG, Q; CHANDAR, Y.J.; CAO, S.; KHARASCH, E. D.; SINGAMANENI, S.; MORRISSEY, J. J. Rapid, Point-of-Care, Paper-Based Plasmonic Biosensor for Zika Virus Diagnosis. **Adv. Biosys**. DOI: 10.1002/adbi.201700096. 2017. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1002/adbi.201700096. Acesso em: 25 Ago. 2019.

JOYCE, M. A.; TYRREL, D. L. The cell biology of hepatitis C virus. **Microbes Infect**, v. 12, p. 263-271, 2010. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26047987. Acesso em: 11 Jan. 2019.

KANG, Q.; YANG, L.; CAI, Q. An electro-catalytic biosensor fabricated with Pt–Au nanoparticle-decorated titania nanotube array. **Bioelectrochemistry**. v. 74, p. 62–65, 2008. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S1567539408001084. Acesso em: 19 Jan. 2019.

KIEW, S. F.; KIEW, L. V.; LEE, H. B.; IMAE, T.; CHUNG, L. C.; Assessing biocompatibility of graphene oxide-based nanocarriers: A review. **Journal of Controlled Release**. v. 226, p. 217-228, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26873333. Acesso em: 19 Jan. 2019.

KOSACK, C. S., NICK, S. Evaluation of two rapid screening assays for detecting hepatitis C antibodies in resource-constrained settings. **Tropical Medicine and International Health,** v. 21, p. 603–609, 2016. Disponível em https://www.ncbi.nlm.nih.gov/pubmed/26945920. Acesso em: 19 Set. 2018.

KRUSS, S.; HILMER, A. J.; ZHANG, J.; REUEL, N. F.; MU, B.; STRANO, M.S. Carbon nanotubes as optical biomedical sensors. **Advanced Drug Delivery Reviews**. v. 65, p. 1933–1950, 2013. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/23906934. Acesso em: 15 Fev. 2019.

LAMEIRA, T. L. M.;RODRIGUES, A. U.; QUEIROZ, M. M. C. Perfil clínico e epidemiológico da hepatite c em rio branco, acre, Brasil. **Rev. Saúde**, v. 9, p. 64 -79, 2013. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/23906889. Acesso em: 12 jan. 2019.

LAUER, Georg M.; WALKER, Bruce D. Hepatitis C virus infection. **New England journal of medicine**, v. 345, p. 41-52, 2001. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/238756. Acesso em: 25 Ago. 2019.

LEE D.; CHANDER Y.; GOYAL SM.; CUI T. Carbon nanotube electric immunoassay for the detection of swine influenza virus h1n1. **Biosensors and Bioelectronics**. v. 5, p. 3482-3487, 2011. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/21354779. Acesso em: 15 Fev. 2019.

LIU, Q.; MA, C.; LIU, X.; WEI, X.; MAO, C.; ZHU, J.; A novel electrochemiluminescence biosensor for the detection of microRNAs based on a DNA functionalized nitrogen doped carbon quantum dots as signal enhancers. **Biosensors and Bioelectronics,** v. 92, p. 273–279, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/21354332. Acesso em: 18 Mar. 2017.

LIU, Y., JIE YU, J., Oriented immobilization of proteins on solid supports for use in biosensors and biochips: a review. **Microchimica Acta**, v.183, p 1–19, 2016. Disponível em:https://www.researchgate.net/publication/281246853_Oriented_immobilization_of_proteins_on_solid_supports_for_use_in_biosensors_and_biochips_a_review. Acesso em: 15 Fev. 2019.

LOWINSOHN, D.; BERTOTTI, M. Sensores eletroquímicos: considerações sobre mecanismos de funcionamento e aplicações no monitoramento de espécies químicas em ambientes microscópicos. **Química Nova**, v. 29, p. 1318-1325, 2006. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-40422006000600029. Acesso em: 12 Jan. 2018.

LU, M., XU, L., ZHANG, X., XIAO, R., WANG, Y. Ag(I)-coordinated hairpin DNA for homogenous electronic monitoring of hepatitis C virus accompanying isothermal cycling signal amplifi-cation strategy. **Biosensors and Bioelectronics**, v. 73, p. 195–201, 2015. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26071691. Acesso em: 12 Jan. 2019.

MARRAZZA, G. Piezoelectric Biosensors for Organophosphate and Carbamate Pesticides: A Review. **Biosensors**, v. 4, p. 301-317, 2014. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4264360/. Acesso em: 18 Fev. 2019.

MAUSS, S., BERG, T., ROCKSTROH, J., SARRAZIN, C. WEDEMEYER, H. Short Guide to Hepatitis C. **Hepatology**, v. 2, p. 1-50, 2014. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4264360/. Acesso em: 11 Fev. 2019.

MEDANY, S. S.; ISMAIL, K. M.; BADAWY, W. A. Kinetics of the electropolymerization of aminoanthraquinone from aqueous solutions and analytical applications of the polymer film. **Journal of AdvancedResearch**, v. 3, p. 261–268, 2012. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4264336/. Acesso em: 14 Mar. 2018.

MEHROTRA, P. Biosensors and their applications – A review. **Journal of oral biology and crânio facial research**, n. 6, p. 1 5 3 – 1 5 9, 2016. Disponível em: https://www.sciencedirect.com/science/article/pii/S2212426815001323. Acesso em: 19 Jan. 2019.

MERICAN, I. et al. Clinical, biochemical and histological features in 102 patients with chronic hepatitis C virus infection. **QJM: An International Journal of Medicine**, v. 86, p. 119-125, 1993. Disponível em: https://www.snfge.org/content/httpwwwsciencedirectcomsciencearticlepiis0016508516300543. Acesso em: 12 Jan. 2019.

MILLONE, M. A. D.; RAMIREZ, E. A.; CHAIN, C. Y.; CRIVARO, A.; ROMANIN, D.; RUMBO, M.; DOCENA, G.; COCCO, M. D.; PEDANO, M. L.; FAINSTEIN, A.; MONTOYA, J.; VELA, M. E.; SALVAREZZA, R. C. SPR Biosensing MUA/Poly-L-lysine Platform for the Detection of 2,4-Dinitrophenol as Small Molecule Model System. **Journal of Nanomaterials**. http://dx.doi.org/10.1155/2016/5432656, 2016. Disponível em: https://www.hindawi.com/journals/jnm/2016/5432656/. Acesso em: 12 Jan. 2019.

MOOSAEI, R., SHARIF, M., RAMEZANNEZHAD, A. Enhancement of tensile, electrical and thermal properties of epoxy nanocomposites through chemical hybridization of polypyrrole and graphene oxide. **Polymer Testing**, v. 60, p.173-186, 2017. Disponível em: https://www.infona.pl/resource/bwmeta1.element.elsevier-12528274-c671-3a20-9ff7-82cc0199b6ae/. Acesso em: 14 Fev. 2019.

MORAES, S. B.; BOTAN, R.; LONA, L. M. F. Síntese e caracterização de nanocompósitos de poliestireno/hidroxissal lamelar. **Quimica Nova**, v. 37, p.18-21, 2013. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-40422014000100004. Acesso em: 19 Mac. 2019.

NYAN, D. C.; SWINSON, K. L. A method for rapid detection and genotype identification of hepatitis C virus 1–6 by one-step reverse transcription loop-mediated isothermal amplification. **International Journal of Infectious Diseases**. v. 43, p. 30–36, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26686938. Acesso em: 31 Mac. 2019.

PACHECO, W. F., SEMAAN, F. S., ALMEIDA, V. G. K., RITTA, A. G. S. L., AUCÉLIO, R. Q. Voltametrias: Uma Breve Revisão Sobre os Conceitos. **Rev. Virtual Quim.,** v. 5 , p. 516-537, 2013. Disponível em: http://rvq.sbq.org.br/detalhe_artigo.asp?id=410. Acesso em: 12 Abr. 2019.

PALAU, W., MASANTE, C., VENTURA, M., PRIMO, C. D., Direct evidence for RNA–RNA interactions at the 3′ end of the Hepatitis C virus genome using surface plasmon resonance. **RNA journal**, v. 19, p. 982–991, 2013. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3683932/. Acesso em: 19 Jan. 2019.

PALMIERI, V.; LAURIOLA, M. C.; CIASCA, G.; CONTI, C.; SPIRITO, M.; PAPI, M. The graphene oxide contradictory effects against human pathogens. **Nanotechnology**. https://doi.org/10.1088/1361-6528/aa6150, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3683932/. Acesso em: 25 Ago. 2019.

PERUMAL, V.; HASHIM, U. Advances in biosensors: principle, architecture and applications. **Journal of Applied Biomedicine**, v. 12, p. 1-15, 2014. Disponível em: https://www.sciencedirect.com/science/article/pii/S1214021X13000082/. Acesso em: 29 Set. 2019.

PESQUERO, N. C. **DESENVOLVIMENTO DE DISPOSITIVOS PARA DIAGNÓSTICO E GENOTIPAGEM DE HEPATITE C.** 2013**.** Tese **(**Doutorado em Química**)** Universidade Estadual Paulista, São Paulo, 2013.

POHANKA, M.; JUN, D.; KUCA, K. Mycotoxin Assays Using Biosensor Technology: A Review. **Drug and Chemical Toxicology**. v. 30, p. 253–261, 2007. Disponível em: https://www.sciencedirect.com/science/article/pii/S1214021X13000082/. Acesso em: 12 Abr. 2019.

RABA, J.; FERNÁNDEZ-BALDO, M. A.; PEREIRA, S. V.; MESSINA, G. A.; BERTOLINO, F. A.; TOSETTI, S.; FERRAMOLA, M. I. S. Analytical biosensors for the pathogenic microrganisms determination. In: MÉNDEZ-VILAS, A. Microbial pathogens and strategies for combating them: science, technology and education**. Ed. FORMATEX**, v 5. p. 227-238, 2013. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3683966/. Acesso em: 22 Jan. 2019.

RAGHWANI, J., ROSE, R, SHERIDAN, I., LEMEY, P., SUCHARD, M. A., SANTANTONIO, T., FARCI, P., KLENERMAN, P., PYBUS, O. G. Exceptional Heterogeneity in Viral Evolutionary Dynamics Characterises Chronic Hepatitis C Virus Infection. **PLOS Pathogens,** v. 15, p. 1-20, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC363687/. Acesso em: 12 Jan. 2019.

RAMBO, M. C. **ESTUDO DA RELAÇÃO ENTRE LIQUEN PLANO BUCAL E HEPATITE C CRÔNICA EM PACIENTES DO HOSPITAL UNIVERSITÁRIO DA UFSC.** 2014. 95 P. Trabalho de conclusão de curso (Bacharelado em Odontologia), Universidade Federal de Santa Catarina.

RASHEED, P. A., N. SANDHYARANI, N. Carbon nanostructures as immobilization platform for DNA: A review on current progress in electrochemical DNA sensors. **Biosensors and Bioelectronics**, v. 97, p. 226–237, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/28601788. Acesso em: 25 Ago. 2019.

ROCKSTROH, J. K., FELD, J. J., CHEVALIEZ, S., CHENG, K., WEDEMEYER, H., SARRAZIN, C., MAASOUMY, B., HERMAND, C., HACKETT, J. COHEN, D. E.DAWSON, G. J. CLOHERTY, G. M., PAWLOTSKY, J. M. HCV core antigen as an alternate test to HCV RNA for assessment of virologic responses to all-oral, interferon-free treatment in HCV genotype 1 infected patients. **Journal of Virological Methods**, v. 245, p. 14–18, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/28601766. Acesso em: 21 Fev. 2019.

SADEGH, H. Development of graphene oxide from graphite: a review on synthesis, characterization and its application in wastewater treatment**. Rev.Adv.Mater. Sci.** v. 49, p. 38-43, 2017. Disponível em: http://www.scielo.br/scielo.php?pid=S0104-66322019000100001&script=sci_arttext. Acesso em: 12 Abr. 2019.

SAHINER, N. Single step poly (L-Lysine) microgel synthesis, characterization and biocompatibility tests. **Polymer**. v. 121, p. 46-54, 2017. Disponível em: https://www.researchgate.net/publication/317488162_Single_step_polyL-Lysine_microgel_synthesis_characterization_and_biocompatibility_tests. Acesso em: 25 Abr. 2018.

SASSOLAS, A., BLUM, L. J., LECA-BOUVIER, B. D. Immobilization strategies to develop enzymatic biosensors. **Biotechnology Advances**, v. 30, p. 489–511, 2012. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/21951558. Acesso em: 29 Jan. 2018.

SHARMA, P. S; PIETRZYK-LE, A.; D'SOUZA, F.; KUTNER, W. Electrochemically synthesized polymers in molecular imprinting for chemical sensing. **Analytical and Bioanalytical Chemistry,** v**.** 402, p. 3177-3204, 2012. Disponível em: https://link.springer.com/article/10.1007/s00216-011-5696-6. Acesso em: 19 Jan. 2019.

SHRIVASTAVA, S.; JADON, N.; JAIN, R.; Next generation polymer nanocomposites based electrochemical sensors and biosensors: a review. **TrAC Trends in Analytical Chemistry.** v. 82, p. 55–67, 2016. Disponível em: https://www.researchgate.net/publication/301742933_Nextgeneration_polymer_nanocomposite-based_electrochemical_sensors_and_biosensors_A_review. Acesso em: 19 Jan. 2019.

SILVA, B. V. M.; RODRÍGUEZ, B. A. G.; SALES, G. F.; SOTOMAYOR, M. D. P.; DUTRA, R. F. An ultrasensitive human cardiac troponin T graphene screen-printed electrode based on electropolymerized-molecularly imprinted conducting polymer. **Biosensors and Bioelectronics**. v. 77, p. 978–985, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26544873. Acesso em: 25 Ago. 2019.

SILVA, M. M .S. et al. Electrochemical detection of dengue virus NS1 protein with a poly(allylamime)/ carbono nanotube layered immunoelectrode. **Journal of Chemical Technology and Biotechnology,** v. 90, p. 194-200, 2015. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26544873. Acesso em: 12 Fev 2019.

SILVA, M. M. S; DIAS, A. C. M. S.; SILVA, B. V. M.; GOMES-FILHO, S. L. R.; KUBOTA, L. T.; GOULART, M. O.; DUTRA, R. A.F. Electrochemical detection of dengue virus NS1 protein with a poly(allylamine)/carbon nanotube layered immunoelectrode. **J Chem Technol Biotechnol,** v.14, p. 23-28, 2014. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/26544897. Acesso em: 12 Jan. 2019.

SILVA, N. M. O.; GERMANO, F. N.; VIDALES-BRAZ, B. M.; ZANELLA, R. C.; SANTOS, D. M.; LOBATO, R.; MARTINEZ, A. M. B. Polymorphisms of IL-10 gene in patients infected with HCV under antiviral treatment in southern Brazil. **Cytokine**, v. 73, p. 253–257, 2015. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/20661604. Acesso em: 12 Jan. 2019.

SILVA, P. M. S.; LIMA, A. L. R.; SILVA, B. V. M.; COELHO, L. B. B.; DUTRA, R. F.; CORREIA, M. T. S. Cratyliamollis lectin nanoelectrode for differential diagnostic of prostate cancer and benign prostatic hyperplasia basedon label-free detection. **Biosensors and Bioelectronics.** v. 85, p. 171–177, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/20661604. Acesso em: 25 Ago. 2018.

SILVA, T. A., MORAES, F. C., JANEGITZ, B. C., FATIBELLO-FILHO, O. Electrochemical Biosensors Based on Nanostructured Carbon Black: A Review. **Journal of Nanomaterials**, v. 17, p. 1-14, 2017. Disponível em: https://www.hindawi.com/journals/jnm/2017/4571614/. Acesso em: 12 Jan. 2019.

SIMHA, A., WEBB, C. M., PRASAD, R., KOLB, N. R., VELDKAMP, P. J. Moral Distress with Obstacles to Hepatitis C Treatment: A Council of Academic Family Medicine Educational Research Alliance (CERA) Study of Family Medicine Program Directors. **JABFM,** v. 31, p. 286 - 291, 2018. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/29535247. Acesso em: 17 Jan. 2019.

SINGHAL, C., INGLE, A., CHAKRABORTY, D., PN, A. K.,C., PUNDIR, S., NARANG, J. Impedimetric genosensor for detection of hepatitis C virus (HCV1)DNA using viral probe on methylene blue doped silica nanoparticles. **International Journal of Biological Macromolecules,** v. 98, p. 84–93, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/29535368. Acesso em: 24 Ago. 2019.

SINGHALA, C., INGLEA, A., CHAKRABORTY, D., KRISHNA, A., NARANG, P. J. Impedimetric genosensor for detection of hepatitis C virus (HCV1)DNA using viral probe on methylene blue doped silica nanoparticles. **International Journal of Biological Macromolecules**, v. 98, p. 84–93, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/28126458. Acesso em: 25 Ago. 2019.

TANAKA, J., AKITA, T., OHISA, M., SAKAMUNE, K., KO, K., UCHIDA. S., SATAKE, M. Trends in the total numbers of HBV and HCV carriers in Japan from 2000 to 2011. **J Viral Hepat**. v. 25, p. 363–372, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/29193549. Acesso em: 12 Ago. 2019

THIRUMALRAJ, B.; KUBENDHIRAN, S.; CHEN, S. M.; LIN, K. Y. Highly sensitive electrochemical detection of palmatine using a biocompatible multiwalled carbon nanotube/poly-L-lysine composite. **Journal of Colloid and Interface Science.** v. 498, p. 144–152, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/29193549. Acesso em: 12 Ago. 2019

TZARUM, N., WILSON, I. A., LAW, M. The neutralizing Face of Hepatitis C virus e2 envelope Glycoprotein. **Frontiers in Immunology,** v. 9, p. 1-8, 2018. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/29193889. Acesso em: 25 Ago. 2019

VANHOMMERIGA, J. W., LAAR, T. J. W. V., KOOT, M., ROOIJEN, M. S. V., SCHINKEL, J., SPEKSNIJDER, A. G. C. L., PRINS, M., VRIES, H. J, BRUISTEN, S. M. Evaluation of a hepatitis C virus (HCV) antigen assay for routine HCV screening among men who have sex with men infected with HIV. **Journal of Virological Methods,** v. 213, p. 147–150, 2015. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/29193578. Acesso em: 12 Jan. 2019

VILLAR, L. M., CRUZ, H. M., BARBOSA, J. R., BEZERRA, C. R., PORTILHO, M. M., SCALIONI, L. P. Update on hepatitis B and C virus diagnosis. **World J Virol**, v. 4, p. 323-342, 2015. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/2866467. Acesso em: 12 Fev. 2019.

WANG, C., JIANG, T., ZHAO, K., DENG, A., LI, J. A novel electrochemiluminescent immunoassay for diclofenac using conductive polymer functionalized graphene oxide as labels and gold nanorods as signal enhancers. **Talanta**, v. 193, p. 184–191, 2019. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/30368289. Acesso em: 12 Ago. 2019.

WANG, L., ZHANG, Y., WU, A., WEI, G. Fabrication of chronocoulometric DNA sensor based on gold nanoparticles/poly(L-lysine) modified glassy carbon electrode. **Analytica Chimica Acta**, v. 985 p. 24-40, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/303682354. Acesso em: 25 Ago. 2019.

WANG, Y.; QU, Y.; YE, X.; WU, K.; LI, C. Fabrication of an electrochemical immunosensor for α-fetoprotein based on a poly-L-lysine-single-walled carbon nanotubes/Prussian blue composite film interface. **J Solid State Electrochem**, v. 20, p. 2217–2222, 2016. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/30368289. Acesso em: 12 Ago. 2019.

WASIK, D.; MULCHANDANI, A.; YATES, M. V. A Heparin-Functionalized Carbon Nanotube-Based Affinity Biosensor for Dengue Virus. **Biosensors and Bioelectronics,** v. 91, p. 811–816, 2017. Disponível em: www.ncbi.nlm.nih.gov/pubmed/28152487. Acesso em: 25 Ago. 2019.

WHO. Hepatitis C. Disponivel em: http://www.who.int/mediacentre/factsheets/fs164/en/ Acesso em 29 de Outubro de 2017. Disponível em: www.ncbi.nlm.nih.gov/pubmed/28152487. Acesso em: 22 Jan. 2018.

WU, L.; YAN, F.; JU, H. An amperometric immunosensor for separation-free immunoassay of CA125 based on its covalent immobilization coupled with thionine on carbon nanofiber. **Journal of immunological methods**. v. 322, p.12-19, 2007. Disponível em: www.ncbi.nlm.nih.gov/pubmed/28152487. Acesso em: 22 Fev. 2019.

ZEN, J.; KUMAR, A. S.; TSAI, D. Recent updates of chemically modified electrodes in analytical chemistry. **Electroanalysis,** v. 15, p. 1073-1087, 2003. Disponível em: www.ncbi.nlm.nih.gov/pubmed/281524358. Acesso em: 12 Fev. 2019.

ZHAN, C.; YU, G.; LU,Y.; WANG, L.; WUJCIKB, E.; WEI, S.; Conductive polymer nanocomposites: a critical review of modern advanced devices. **Journal of Materials Chemistry C.** v. 5, p. 1569—1585, 2017. Disponível em: https://pubs.rsc.org/en/content/articlelanding/2017/tc/c6tc04269d#!divAbstract. Acesso em: 19 Jan. 2019.

ZHU, Z.; GARCIA-GANCEDO, L.; FLEWITT, A. J.; XIE, H.; FRANCIS MOUSSY, F.; MILNE, W. I. A Critical Review of Glucose Biosensors Based on Carbon Nanomaterials: Carbon Nanotubes and Graphene**. Sensors**. *D*oi:10.3390/s120505996, 2012. Disponível em: https://www.ncbi.nlm.nih.gov/pubmed/22778628. Acesso em: 28 Abr. 2019.

APENDICE A - Point-of-Care Electrochemical Immunosensors Applied to Diagnostic in Health

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Advances in Biosensors: Reviews, 2018

2.1. Introduction

Point-of-care testings (PoCT) have been recognized as one of the most attractive methods for decentralization of analytical practices, being mainly developed to diagnostics that require rapid interventions, such as cardiovascular diseases, drug intoxication, emergency preparedness in surgical procedures, containment of transmissibility, spread of infectious diseases, and surveys in endemic or epidemic outbreaks [1]. Another interesting application of PoCTs devices is in continuous monitoring of markers that require recurrent evaluations, glicemy or mostly in therapies and prolonged treatments of diseases like the cancer, being also benefit for treatment and monitoring of patients that live in areas far from central laboratories. In these situations, the conventional laboratorial testings become impracticable, since samples should be transported, processed and results returned to the doctors. Challenges in developing of PoCs for medical diagnostics involve to combine the advantages of fast results, low cost and user friendly processing, without loss of diagnostic sensibility and specificity, when they are compared to laboratorial analyses.

PoCTs are analytical devices designed to be used near the bedside, reducing the turnaround time of the diagnostic cycle, being usually rapid (up to 30 minutes). PoCT are processed outside hospital or laboratory that do not require skilled personnel for managements [2]. Currently, PoCT tests are considered practical and economical methods, being nowadays considered as one of the most attractive analytical possibilities, compared to the chemical analysers, immunoanalyzers, PCR (polymerase chain reaction) and others analysers [2]. Among PoCT devices, lateral flow assays (LFA) and biosensors addressed to immunoassays are more economically profitable than enzymatic assays, especially regarding to laboratorial analyses.

Lateral flow assays (LFA) based on immunochromatographic tests are paper assays that use immobilized antibodies or antigens to capture target analytes in samples. A color band resulted from molecule (antigen or antibody) or material labeling reveals this reaction, usually supplying qualitative results. However, additional image resources can be used to produce quantitative data based in contrast and brightness of color band [3]. A typical LFA is formed by overlapping membranes mounted on an inert rigid support, which confers stability and facilitates handling of the test [4]. The tip of the strip has

a sample pad made of adsorbent material, where the sample is applied. The samples are transported by capillarity to the conjugation pad containing the labeled antibodies for biorecognition. The interaction between the target analytes and these antibodies form complexes that migrate to the reaction zone, usually formed by a nitrocellulose membrane. In this zone, there are two lines of immobilized antibodies, one to the target molecule and the control to define the results [8]. A schematic design of a LFA is shown in Fig. 2.1. On the last few decades, advances of nanotechnology has allowed incorporation of gold nanoparticles to LFA, improving the sensitive of the analytical testings [7] Currently, LFA immunoassay have been developed to several applications, allowing the screening of infectious diseases (HIV, viral hepatitis, tuberculosis and herpes simplex virus and others)[8]. LFA has also been applied to point-of-care detection of cardiac markers such as troponin, H-FABP, hepatitis and others, possibility a semi-quantitative analysis, however it is quite limited, because the results are color band-dependents, thus the results are subject of human error of interpretation [9].

In attempting to overcome the limitations denoted by LFA, contrary, immunosensors supply a quantifiable signal, and the amount of analyte detected are proportional to the analyte concentrations, independently of detected species: antibodies, antigens, enzymes, or other chemical species. The interest for immunosensors has been exponentially growing on the last decades due to combine advantages of high sensitivity, user-friendly processing and portability, beside to present a low cost per analyses (Fig. 2.2)

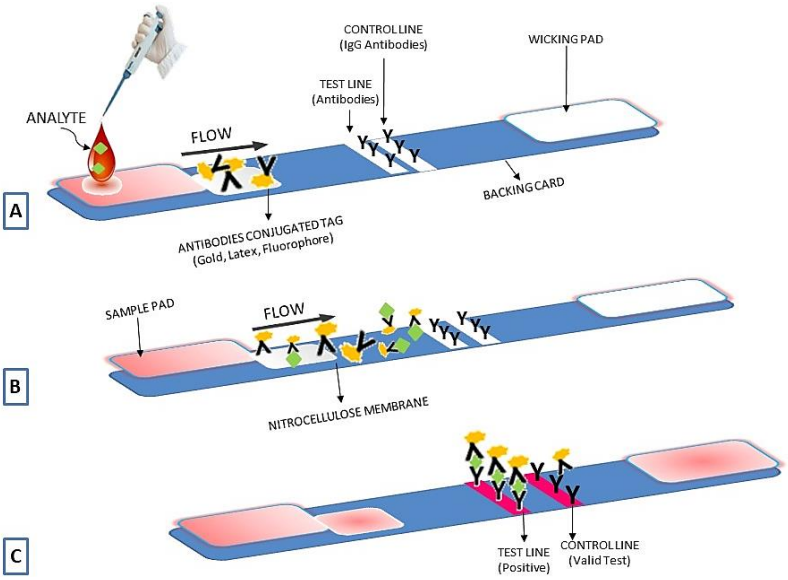

Fig. 2.1. Schematic design of Lateral Flow Imunoassay at different steps: (A) adding the samples containing antigens and immunoglobulins; (B) migration of antigens complexed with labelled antibodies, and (C) immunocomplex formed and immunoglobulis are positioned by affinity on the paper regions where labelled antibodies are exhibited by a color band.

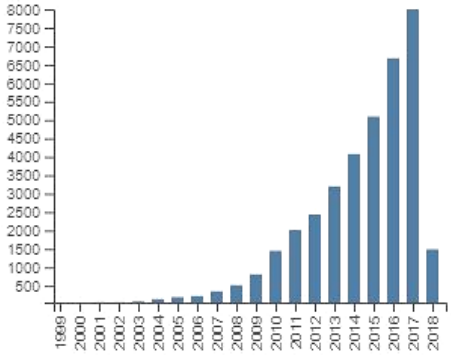

Fig. 2.2. Number of citation over the last decades (Extracted from Web of Science base: [“immunosensor” OR “electrode” OR “biosensor”] AND [“point-of-care”] in March 2018).

2.2. PoCT Electrochemical Immunosensor

Immunosensors are based on the specific antigen-antibody interactions causing a pertubation on the electrode surface by molecule capturing by immobilized antigens or antibodies; this pertubation is converted into measurable signals by a transducer. In general, the signal is amplified, processed and readout in output display [10]. Specificity of immunosensors is mainly dependent of affinity between antigen-antibody. Monoclonal and polyclonal antibodies can be used in immobilization technique developments, nevertheless monoclonal antibodies are more attractive due to recognize only a one epitope of an antigen, being more specific, although commonly have a higher cost [11] Recombinant antigens have been more recently used to produce antibodies with more selectively, in order to recognize only one epitope region.

Screen-printed electrode (SPE) has significantly contributed for PoCT developments. The layer-by-layer printing of commercial or self-made inks onto different types of rigid and flexible substrates. Conventionally, SPE comprises one sensing unit with three printed electrodes, including a working electrode, a counter electrode and a reference electrode. The composition of the inks chosen in the printing process is essential to the selective determination intended for each analysis [12]. Commonly, SPE uses voltammetric techniques, measuring changes on current responses produced by a controlled potential (constant or periodic). Current responses are generated by diffusion of redox species from electrolyte/electrode interface, being proportional to the binding events, i.e. antigens or antibodies captured.

Innumerous point-of-care immunosensors using amperometric transduction have been developed for clinical diagnostics, such as for HIV [13], prostate specific antigen (PSA) [14], celiac disease [15], cardiac troponin T [16] and cardiac troponin I [17]. Other transducer types using the SPE have also been described for impedance [18] or capacitance [19] measurements.

Recent advances in the SPE development for clinical diagnostic were obtained with progress derived from synthesis of nanostructured electrode surfaces. Metallic nanoparticles, nanowires, carbon nanotubes, graphene, and their respective nanocomposites have been widely used, in either pastes, or forming film on the working electrodes [20]. nanocomposites or nanofilms was possible to increase the amount of immobilized biomolecules by the working electrode area increases. Additionally, nanomaterials have also contributed to

increase the sensitivity and selectivity of sensors, due to their electrical, optics, acoustic and other interesting proprieties, particularly indispensable and individuals of each nanomaterial that is capable to produce devices with more reproducible results and robustness [21].

2.3. Advances on PoCT Immunosensors

Nanomaterials have improved the efficiency and reliability of electrochemical PoCT immunosensors, allowing a lower limit of detection in the concentrations of antigens or antibodies present in biologic fluid samples that was not possible. Nanomaterials can be defined based on size parameter(s), being under 100 nm sized in, at least, one dimension. Commonly, in nanoscalle, these materials present new properties that are not normally observed, when they are in bulk. These alterations are obtained by the quantum effects of size, being especially evident in carbon allotropes and metal nanoparticle [22, 23]. For this reason, it is clear that the progress of bioanalytical assays will rely heavily on innovations in nanotechnology [24, 25].

Several nanomaterials have contributed to electrochemical immunosensor developments, among them metal nanoparticles, metal oxides nanoparticles, carbon nanotubes, graphene, their corresponding nanocomposites and quantum dots are more commonly employed (Fig. 2.3) [26].

2.3.1. PoCT Immunosensors Based on Carbon Allotropes

Recently, the contribution of carbon allotropes in the construction of electrochemical immuno-PoCTs has gained prominence due to the small size of the carbon atoms and the number of electrons they can share, allowing the formation of several bonding patterns and stable versatile materials with excellent intrinsic properties such as electrical conductivity, large surface area, ease of functionalization and biocompatibility [27].

Carbon nanotube

Among the nanostructures synthesized from carbon allotropes we can highlight the nanotubes, which were discovered in 1991 by Iijima, enabling interaction with biomolecules for biosensor applications [28]. Carbon nanotubes (CNT) can be described as hexagonal arrangements in cylindrical format, held by Van der Waals interactions in the adjacent layers. They promote rapid electron transfer, increasing the reaction rate of many electroactive species, and then decreasing the electrode response time of the Immuno-PoCTs, thereby achieving high sensitivity with low detection limits [29]. With respect to the structure the CNT can be classified in two forms: single wall nanotubes (SWCNT), formed by a single layer of carbon atoms arranged in a hexagonal way, and multiple wall nanotubes (MWCNT), which consists of multiple layers of carbon atoms arranged in a hexagonal way arranged around a central area. The length of the CNT can range from nanometers to centimeters, but the diameter varies in the order of nanometers, depending on the type of CNT [30].

Activation or functionalization of CNTs by oxidation treatment introduces chemical functional groups, including alcoholic, carboxylic, aldehydic, ketonic, and esteric oxygenated functional groups [31]. These groups allow a greater interaction between CNTs and antibodies, enabling them to immobilize more molecules on the sensor surface facilitating non-random binding and exposing their binding sites or antigenic regions to their target analytes (Fig. 2.4) [32].

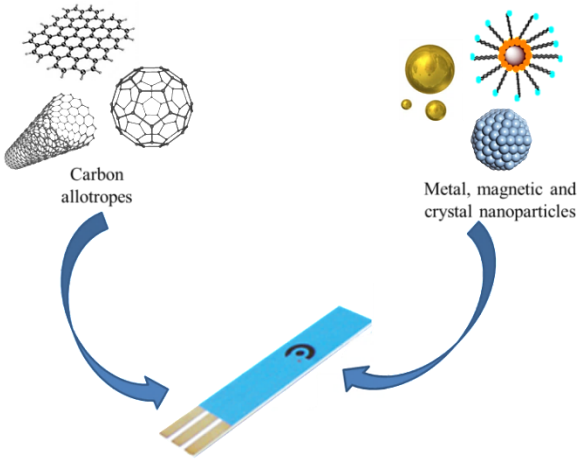

Fig. 2.3. Nanomaterials with potential application in PoCT device. Nanomaterials can works as carriers or reporters for signal generation or powerful amplification in the transduction systems. Their nanobioapplication in SPE has been very promising.

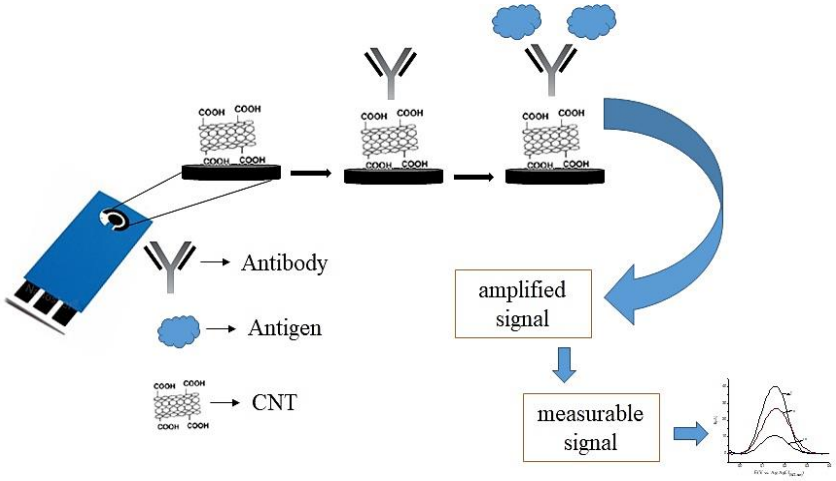

Fig. 2.4. Use of CNTs to detect the interaction between antigen-antibody in PoCT.

Studies have shown that CNTs interact with other materials, improving the intrinsic qualities of the PoCTs immunosensor. Dias et al. [33], (2013) produced a dengue virus (non-structural protein 1) immuno-PoCT based on a homogeneous mixture consisting of carboxylated carbon nanotubes dispersed in carbon paint as a printed working electrode. The effect of the matrix, as well as the performance of the assays, was successfully evaluated using the spiked blood serum sample, obtaining excellent recovery values in the results. The carbon nanotubes incorporated into the carbon paint improved the reproducibility and sensitivity of the CNT-SPE immunosensorIn another work, Silva et al. [34] (2013) developed a label-free immunosensor based on printed electrodes for carbon nanotubes functionalized with amine groups to detect cardiac troponin. PoCT was developed by the homogenization between the carbon paint and the amine carbon nanotubes on a polyethylene terephthalate substrate for thin films. The use of carbon nanotubes increased the reproducibility and stability of the sensor, and the amine groups allowed the non-random immobilization of antibodies against cardiac troponin T.

Aiming to allow the orientation of Carbon Nanotubes in the detection surface, polymer films have been commonly employed. The polymers interact with the CNT through the functional groups
(-COOH, -OH, -NH_2) and may form nanocomposites or nanohybrids. As examples we can cite the work of Sanchez-Tirado et al. [35], (2017) where dual screen-printed carbon electrodes modified with 4-carboxyphenyl-functionalized double-walled carbon nanotubes were used for the preparation of electrochemical immunosensors for the simultaneous determination of the cytokines Interleukin-1β (IL-1β) and factor necrosis tumor α (TNF-α). In addition, the dual immunosensor exhibits excellent reproducibility of the measurements, storage stability and selectivity as well as negligible crosstalking. In recent years, studies have shown that the use of CNT combined with conductive polymers can improve sensitivity and increase electron transfer on the sensor platform. In another study Gomes et al. [36], (2013) produced a nanostructured SPE immunosensor based on carbon nanotubes supported by a conductive polymer film for detection of cardiac Troponin T (cTnT). The combined use of polyethyleneimine (PEI) film and CNT provided important advantages for obtaining a highly sensitive analytical method for cTnT.

Graphene

Another prominent carbon nanomaterial is graphene (G). It is a 2D material of atomic thickness, formed by carbon atoms with sp2 hybridization, forming a structure of hexagonal shape similar to a hive. The characterization and identification of graphene was first performed in 2004, through successive stages of graphite exfoliation using commercial adhesive tapes [37]. Among its remarkable properties, we can cite transparency of the sheets (optical transmittance of ~97.7 %) and large surface area (2630 m^2 g^{-1}). Furthermore, it is a good heat conductor (thermal conductivity of 500 W m^{-1} K^{-1}), chemically inert and a semimetal with high electron transfer (charge mobility of 250 000 cm^2 V^{-1} s^{-1} in room temperature) [38].

According to its physical and chemical characteristics we can find several forms of graphene, among them Graphene Oxide (GO) and Reduced Graphene Oxide (RGO), which, because of their particularities, are highly attractive for the assembly of sensor surfaces [39]. GO has two dimensions, consisting of an hexagonal network of Sp2 bonds between carbon atoms (CC) and by Sp3 bonds with oxygen atoms (CO) forming carboxyl groups (-COOH), hydroxyls (-OH) or epoxy (-O-). This makes GO an excellent material for biological applications, since its functional groups readily interact with nucleic acids, proteins, cells, and other organic molecules [40]. Yukird et al. [41], (2017) developed an electrochemical immunosensor based on a nanohybrid formed by graphene and polyaniline (G/PANI). Electrospraying of G/PANI increased the electrode surface area while electropolymerization of aniline increased the number of amino groups (-NH_2) for antibody immobilization.

Reduced graphene oxide is another material that has been widely used in immunoelectrochemical analysis, due to its high effective surface area and high electrical conductivity [42]. It is produced from reduction of GO via thermal, chemical, electrochemical and laser-scribing methods. In RGO synthesis, functional groups are removed and the conductivity is increased again [40]. As examples of the use of graphene for the production of Immuno-PoCTs we can mention the work of Silva et al., (2016) [43], that produced a biomimetic sensor for the detection of Troponin T based on a nanocomposite formed by the conjugation of RGO and Polipyrrole. Another example is Lee et al. [44] (2017), who developed an electrochemical immunosensor for the detection of carcinoembryonic antigen.

In this method, silver nanoparticles were mixed with RGO to modify the surface of screen-printed carbon electrode.

2.3.2. PoCT Immunosensors Based on Metal Nanoparticles

Metal nanomaterials have been aroused interest due to their special optical and electrocatalytic properties. They are often incorporated by adhesion or binding to the robustly modified transduction platforms. Fantastic devices with enhanced capabilities for health applications can be fabricated by the assembly of nanoparticles and immunocomponents [45]. In addition to maintain the bioreceptors, metal nanoparticles can work as electronic conduction vehicles in electrochemical biosensors, which allow electrons produced in bioreactions to be transported to sensing electrodes or convert other physiochemical changes to measurable signals that are proportional to the analyte concentration [46]. Metal nanoestructures, semiconductor nanoparticles and metal oxide nanostructures have been considered as potential signal labels when attached to secondary antibodies to stimulate the development of signal amplification strategy for immunosensing [47].

Gold nanoparticles

Gold is an inert metal in macroscale, but gold nanoparticles (GNPs) are adopted nanomaterial often explored as detectable labels to enhance a suitable signal, thereby providing an intense, pronounced and vivid mark. The color change of GNPs are observable with bare eye. This optical property of revelation in visible color is valuable especially in colorimetric assays [48]. Although they have a higher cost, they present high conductivity, excellent biocompatibility, superior stability, low toxicity, relatively simple production and modification [49]. Thus, colloidal GNPs have been used to modify solid electrodes and has shown advantages in feasibly attachment of the immunological molecules and the electron transfer that increase the electrochemical signals. The strong affinity for the amino groups is explored and gold provide a microenvironment compatible with biomolecules, remaining their activity even after immobilization [50]. Moreover, the formation of self-assembled monolayers (SAMs) through oriented Au-S bonds affords great attention to gold toward SPEs for adhesion of more components. Gold SPE helps to deposit antibody in close vicinity with transducer and GNPs help to cast antibody in close vicinity with the antigen and hence results in the increase of sensitivity until femtogram level [51]. Also, Jacobs and coworkers [52] have proposed an 119immunosensors for ultrasensitive detection of troponin-T based on antibody conjugated to GNPs. Using electrochemical impedance spectroscopy, the interdigitated sensor was able to detect concentrations in femtogram per milliliter (fg/mL) of this cardiac marker. Recently, Sabouri et al. [53] have developed a sensitive 119mmunosensors for detection of Hepatitis B virus based on GNPs. HbsAg was targeted by a primary antibody and a secondary antibody co-immobilized on luminol-GNPs, with detection limit of 14 pg/mL.

Silver nanoparticles

Silver is a relatively cheap noble metal that exhibit superior properties over gold on the nanoscale, mainly of optical nature [54]. Its optical profile exhibits the sharpest and most intense bands among metals [55]. Consequently, for convenience, colorimetric assays are prevalent with silver nanoparticles (SNPs) by the straightforward color change discrimination. They can be oxidized more easily and offer improved electrochemical activity, making them good candidates for detection tags in electrochemical sensing. The utilization either naked or conjugated with recognition probes as signal transduction elements for analyte detection in biosensors was shown to improve the detection limits and enhance their diagnostic

performance [56]. For this, silver nanostructures need to be associated with recognition molecules that can selectively detect and capture the analyte of interest. However, the functionalization still is a challenging process. They are less stable in aqueous dispersions and are susceptible to oxidation and etching by chloride ions. By their limited stability and difficulty to functionalize, SNPs have become less popular [57]. Considering practical situations, Hao et al. [58] have developed a direct electrochemical detection approach to assay generically proteins by using SNPs labels coupled covalently with antibody on a SPE. The detection limit found was 0.4 ng/mL. Now, Felici and colleagues [59] have described a novel prototype of label-free 120mmunosensors using SPE and exploiting SNPs as a backing material and electrochemical tracker. Che and coworkers [60] have constructed an amperometric 120mmunosensors for the determination of α-1-fetoprotein, a tumor marker found in several malignant diseases. Multiwalled carbon nanotube-silver nanoparticle composite modified on the surface of a glassy carbon electrode leading a detection limit of 0.08 ng/m. Similarly, Ibupoto and colleagues [61] have described a new potentiometric 120mmunosensors for the selective detection of d-dimer using SNPs decorated ZnO nanotubes anchored to antibodies. D-dimer is a biomarker found at high levels in deep vein thrombosis disorders. It was found a detection limit of 1.00×10^{-6} μg/mL.

Magnetic nanoparticle

Comparatively, magnetic nanoparticles (MNPs) are cheaper to produce, being considered physically and chemically stable, biocompatible and environmentally safe. Magnetic labels have certain peculiarities for biosensing applications, like absence of preprocessing stage for sample purification, since biological entities do not show any magnetic behavior or susceptibility and therefore, no interferences or noise is to expect during signal capturing [62]. Hence, they are also important items for biomedical applications involved in LFA systems as a colored reagent, possessing strong brown coloration. One promising utility is magnetic preconcentration before the detection event. MNPs conjugated to bioreceptor unit can simply be mixed in solution to interact specifically with the analyte. They offer the convenience of separation via external magnetic field, permitting them easily be attracted with a small magnet, losing their magnetic effect when the field is removed. This way, these nanoparticles can be efficiently separated and isolated from the solution [56]. However, the main strategy is the integration of MNPs into the transducer element or the modification of the sensor surface. Despite a wide range of ferromagnetic materials, iron oxides (Fe_2O_3 and Fe_3O_4) are most commonly used for generation or amplification of analytical signal [63]. Employing proper functionalization methods, some notable benefits are achieved such as rapid analysis process, better stability and low detection limit. Besides, they are fluorescent alternatives that offer ease of handling, low production cost and smaller size of final fabricated device when compared to fluorophores [64]. For instance, combining the aforementioned trends, a novel amperometric magnetoimmunoassay based on MNPs pulled by magnetic field on the screen-printed carbon electrodes surface was developed for the selective determination of Legionella pneumophila. The achieved limit of detection by Martín et al. was 104 Colony Forming Units (CFUs)/mL [65]. Singh and Krishnan achieved the first serum insulin voltammetric immunosensor for clinical diagnosis of type 1 and type 2 diabetic disorders. It was reported a lower detection limit of 5 pM for free insulin present in serum using functionalized magnetite nanoparticles [66].

Metal oxide nanoparticles

Zinc oxide (ZnO) also belong group of elite nanomaterials with inherent optical, and piezoelectric properties. It is a semiconducting material that exhibits biomimetic, high catalytic efficiency, little toxicity, low biodegradability, and stable immobilization of proteins due to high isoelectric point without distorting their bioactivity [67]. Beside good electron transfer, this metal oxide nanoparticle denotes a strong adsorption capability, offering numerous sites to antibodies, enzymes and proteins which make them choice for biosensors. It should be conjugated with biological molecules without losing the integrity [68]. For example, a glucose electrochemical sensor based on ZnO nanorods was investigated by Marie and coworkers [69]. The lower limit of detection was 0.22 μM. And a microfluidic immunosensor applied in congenital hypothyroidism screening was presented by Seia and colleagues [70]. ZnO nanobeads were employed as platform for monoclonal antibody immobilization to specifically capture thyrotropin hormone. The electrochemical detection limit of glass microchip was 0.00087 μUI mL.

2.4. Lab-On-A-Chip Based Immunosensors

Due to the in-depth knowledge of nanomaterials, great advances were achieved, making it possible to implement confined labs on a single chip or laboratory analysis system. Lab-on-a-chip combines analysis, reaction and processing in a single microchip, i.e., the ability to gather multiple key functions of a size reduced laboratory on an electronic device with a few square centimeters, which typically manipulates human fluids in the order of microliters to nanoliters [26]. This approach have been extensively applied in point of care devices due to advantages such as compactness, mobility, integrability, modularity, reconfigurability, embedded computing, limited power consumption and minimum need to sample and reagent when enormous amounts of volume are not available [71]. Additionally, lab-on-chip platforms are hermetically enclosed with precise control conditions, avoiding evaporation and minimizing the risk of contamination by potentially infectious biological specimens [72]. Regarding personalized healthcare, the multiple detection by a single PoCT is an important trend which could replace time-consuming laboratory analyses [73]. In addition to releasing results in minutes, they play an important role in management and early investigation of diseases and outbreaks [74]. One of the purposes is the development of a chip-based, miniaturized and portable system that allows for the assay of different analytes in complex samples. In this way, many researches in the scientific community have focused on paper-based and printed electrode technologies as approaches for fabricating these diagnostic systems. These technologies are affordable, user-friendly, rapid, and scalable for manufacturing. Moreover, the association with nanomaterials provides a path for the development of highly sensitive and selective biosensors for prospective generation POC tools [21].

Paper-based microfluidics or lab on paper is a novel system for handling and analysis of fluid extracellular for a variety of medical applications, such as healthcare and screening [75]. This technology presents simplicity, portability, disposability, low-cost and allows the automation of multi-step processes [76]. Nitrocellulose membrane, chromatography paper and filter paper are attractive substrates for fabricating microfluidic device, because they are natural, porous, ubiquitous and inexpensive materials. Confining solvents and reagents in specific points, paper can drive and regulate aqueous movement passively using capillary forces without supplying of some kind of external energy, and the migration perform the sorting, mixing and uniform separation of the liquid samples diffused [77]. Furthermore, the chemical

composition of paper permits the covalent bonding of bioactive compounds onto the surface. On the other hand, some obstacles to become an ideal PoCT are liquid evaporation, sample retention and nonspecific adsorption. These adversities could lead to false response errors and decreased sensitivity [21]. Its mode of construction is creating a set of microchannels bounded by hydrophobic barriers patterned on paper substrates witch the flow is conducted within the hydrophilic channels and consequently, fluid can be coordinated of a controlled mode. Two-dimensional (2D) and three-dimensional 3D microfluidic channels have been already built on paper, being able to transport biological liquids injected separately by pathways for performing assays and quantifying concentrations of distinct analytes [78]. Printing is the one of the most commonly used techniques to achieve minimal consumption of hydrophobic material [79]. A wave of advancements in 3D printing technology to simplify in agile designing and fabrication supports the durability, flexibility and performance of PoC microfluidic [80].

Different detection methods have been employed for a semi-quantitative detection, analytical assays based on colorimetric method, the results can be visually verified to the unaided eye or interpreted by a reader [81]. Nevertheless, fluorescence or electrochemical methods have become more widespread and attractive because of their high accuracy, sensitivity and lower limit of detection. Further, electrochemistry is less subject to the interference compounds exposed in the biological specimens, because it is not affected by ambient lighting conditions [21]. Colorimetric revealing has been expansively applied due to its simplicity and compatibility with cameras. Mobile phones are accessories widely available, allowing be coupled, and so, they are very suitable for incorporation into portable microfluidic devices. Their rapid improvement of hardware and software, high-resolution cameras, processing power and worldwide coverage of wireless internet network connection can facilitate diagnostic access, permit continuous monitoring of health parameters and promote increased surveillance notifications. This way, it is possible to do geo-timed reports and tracking of data automated providing governments with statistical information for clinical and epidemiological impact evaluation and counter-measures policies implementation. In fact, 3D printers and smartphones are instruments that are revolutionizing the future of lab-on-chip platform [82].

Other innovative actuation principle is centrifugal microfluidic that taking advantage of the forces acting on liquids in rotating chips. A spindle motor is necessary to press the fluid in the microfluidic chip. The centrifugal systems are particularly important for tasks involving separation of particles in suspension, as even small differences in density between solid part and surrounding medium will result in sedimentation. It allows to perform the fluid manipulation within operational cartridges without the need of external micropumps and microvalves or previous sample preparation, as in the case to extract cell free plasma from whole blood [83]. Many challenges have been solved requiring only little of user interaction. These emergent microfluidic systems with integrated sensing, also termed lab-on-a-disc, are typically based on optical techniques, for example, absorbance, fluorescence or imaging. Optical readout with movable instrumentation are a successful detection and ensures several advantages: non-contact, high sensitivity, and the availability of optical components such as lasers and photo detectors or even constituents developed for optical disc drives [84].

2.5. Conclusions

Although great advances have been achieved in the development of PoCT immunosensors applied to health that facilitated the diagnosis of many diseases, control and handling more

effectively, allowing analysis or multi-analysis more quickly, more remains to be done to make PoCT a practical devices for clinical routine. Carbon nanotubes, graphene metallic and magnetic nanoparticles nanostructures are examples of nanomaterials that have been widely used for electrochemical PoCTs, improving the amperometric transductions by promoting increase on the electron transfer and offer better electrocatalytic activity. Additionally, due to the large superficial area of nanomaterials, they are able result in increase on electroactive surface area, implying a high sensitivity for PoCTs. While many challenges still need to be overcome, the focus on PoCT immunosensor researches have grown exponentially on the last few decades. Many advantages make them ideal analytical methods: the phlebotomy step is avoided and replaced by a simpler and safer procedure; the collection of capillary blood with a few microliters can be performed on bedside; turnaround time of the diagnostic cycle is dramatically reduced and the results can be immediately informed to the patient, possibiliting the decentralization of outpatient services; and also the coupling with technologies for mobile phones and similar devices is possible.

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References

[1]. E. C. Rama, A. Costa-García, Screen-printed Electrochemical Immunosensors for the Detection of Cancer and Cardiovascular Biomarkers, *Electroanalysis*, Vol. 28, Issue 8, Aug. 2016, pp. 1700-1715.

[2]. C. Florkowski, A. Don-Wauchope, N. Gimenez, K. Rodriguez-Capote, J. Wils, A. Zemlin, Point-of-care testing (POCT) and evidence-based laboratory medicine (EBLM) – does it leverage any advantage in clinical decision making?, *Crit. Rev. Clin. Lab. Sci.*, Vol. 54, Issue 7-8, November 2017, pp. 471-494.

[3]. X. Fu, Z. Cheng, J. Yu, P. Choo, L. Chen, J. Choo, A SERS-based lateral flow assay biosensor for highly sensitive detection of HIV-1 DNA, *Biosens. Bioelectron.*, Vol. 78, 2016, pp. 530-537.

[4]. K. M. Koczula, A. Gallotta, Lateral flow assays, *Essays Biochem.*, Vol. 60, Issue 1, 2016, pp. 111-120.

[5]. M. Sajid, A. N. Kawde, M. Daud, Designs, formats and applications of lateral flow assay: A literature review, *J. Saudi Chem. Soc.*, Vol. 19, Issue 6, 2015, pp. 689-705.

[6]. E. B. Bahadır, M. K. Sezgintürk, Lateral flow assays: Principles, designs and labels, *TrAC – Trends Anal. Chem.*, Vol. 82, 2016, pp. 286-306.

[7]. D. Quesada-González, A. Merkoçi, Nanoparticle-based lateral flow biosensors, *Biosens. Bioelectron.*, Vol. 73, Nov. 2015, pp. 47-63.

[8]. J.-H. Lee, *et al.*, Multiplex diagnosis of viral infectious diseases (AIDS, hepatitis C, and hepatitis A) based on point of care lateral flow assay using engineered proteinticles, *Biosens. Bioelectron.*, Vol. 69, July 2015, pp. 213-225.

[9]. X. Gong, *et al.*, A review of fluorescent signal-based lateral flow immunochromatographic strips, *J. Mater. Chem. B*, Vol. 5, Issue 26, July 2017, pp. 5079-5091.

[10]. C. Kokkinos, A. Economou, M. I. Prodromidis, Electrochemical immunosensors: Critical survey of different architectures and transduction strategies, *Trends Anal. Chem.*, Vol. 79, May 2016, pp. 88-105.

[11]. N. S. Lipman, L. R. Jackson, L. J. Trudel, F. Weis-Garcia, Monoclonal versus polyclonal antibodies: Distinguishing characteristics, applications, and information resources, *ILAR J.*, Vol. 46, Issue 3, January 2005, pp. 258-268.

[12]. R. A. S. Couto, J. L. F. C. Lima, M. B. Quinaz, Recent developments, characteristics and potential applications of screen-printed electrodes in pharmaceutical and biological analysis, *Talanta*, Vol. 146,

January 2016, pp. 801-814.
[13]. N. Gan, X. Du, Y. Cao, F. Hu, T. Li, Q. Jiang, An Ultrasensitive electrochemical immunosensor for HIV p24 based on Fe_3O_4@SiO_2 nanomagnetic probes and nanogold colloid-labeled enzyme-antibody copolymer as signal tag, *Materials (Basel).*, Vol. 6, Issue 4, March 2013, pp. 1255-1269.
[14]. L. Suresh, P. K. Brahman, K. R. Reddy, J. S. Bondili, Development of an electrochemical immunosensor based on gold nanoparticles incorporated chitosan biopolymer nanocomposite film for the detection of prostate cancer using PSA as biomarker, *Enzyme Microb. Technol.*, Vol. 112, May 2018, pp. 43-51.
[15]. M. M. P. S. Neves, M. B. González-García, H. P. A. Nouws, A. Costa-García, Celiac disease detection using a transglutaminase electrochemical immunosensor fabricated on nanohybrid screen-printed carbon electrodes, *Biosens. Bioelectron.*, Vol. 31, Issue 1, January 2012, pp. 95-100.
[16]. B. V. M. Silva, B. A. G. Rodríguez, G. F. Sales, M. P. T. Sotomayor, R. F. Dutra, An ultrasensitive human cardiac troponin T graphene screen-printed electrode based on electropolymerized-molecularly imprinted conducting polymer, *Biosens. Bioelectron.*, Vol. 77, 2016, pp. 978-985.
[17]. Y. Xu, S. Yang, W. Shi, Fabrication of an immunosensor for cardiac troponin I determination, *Int. J. Electrochem. Sci.*, Vol. 12, Issue 9, 2017, pp. 7931-7940.
[18]. A. Afkhami, P. Hashemi, H. Bagheri, J. Salimian, A. Ahmadi, T. Madrakian, Impedimetric immunosensor for the label-free and direct detection of botulinum neurotoxin serotype A using Au nanoparticles/graphene-chitosan composite, *Biosens. Bioelectron.*, Vol. 93, Jul. 2017, pp. 124-131.
[19]. E. A. de Vasconcelos, N. G. Peres, C. O. Pereira, V. L. da Silva, E. F. da Silva, R. F. Dutra, Potential of a simplified measurement scheme and device structure for a low cost label-free point-of-care capacitive biosensor, *Biosens. Bioelectron.*, Vol. 25, Issue 4, December 2009, pp. 870-876.
[20]. Z. Taleat, A. Khoshroo, M. Mazloum-Ardakani, Screen-printed electrodes for biosensing: A review (2008-2013), *Microchim. Acta*, Vol. 181, Issue 9-10, July 2014, pp. 865-891.
[21]. L. Syedmoradi, M. Daneshpour, M. Alvandipour, F. A. Gomez, H. Hajghassem, K. Omidfar, Point of care testing: The impact of nanotechnology, *Biosens. Bioelectron.*, Vol. 87, January 2017, pp. 373-387.
[22]. M. Vidotti, R. Torresi, S. I. Córdoba De Torresi, Eletrodos modificados por hidróxido de níquel: Um estudo de revisão sobre suas propriedades estruturais e eletroquímicas visando suas aplicações em eletrocatálise, Eletrocromismo E baterias secundárias, *Quim. Nov.*, Vol. 33, Issue 10, 2010, pp. 2176-2186.
[23]. Q. Huang, *et al.*, Nanotechnology-based strategies for early cancer diagnosis using circulating tumor cells as a liquid biopsy, *Nanotheranostics*, Vol. 2, Issue 1, 2018, pp. 21-41.
[24]. Z. Farka, T. Juřík, D. Kovář, L. Trnková, P. Skládal, Nanoparticle-based immunochemical biosensors and assays: Recent advances and challenges, *Chem. Rev.*, Vol. 117, Issue 15, August 2017, pp. 9973-10042.
[25]. N. J. Wittenberg, C. L. Haynes, Using nanoparticles to push the limits of detection, *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology*, Vol. 1, Issue 2, March 2009, pp. 237-254.
[26]. K. S. Krishna, Y. Li, S. Li, C. S. S. R. Kumar, Lab-on-a-chip synthesis of inorganic nanomaterials and quantum dots for biomedical applications, *Adv. Drug Deliv. Rev.*, Vol. 65, Issue 11-12, November 2013, pp. 1470-1495.
[27]. Z. Wang, Z. Dai, Carbon nanomaterial-based electrochemical biosensors: An overview, *Nanoscale*, Vol. 7, Issue 15, 2015, pp. 6420-6431.
[28]. A. Ghasemi, *et al.*, Carbon nanotubes in microfluidic lab-on-a-chip technology: Current trends and future perspectives, *Microfluid. Nanofluidics*, Vol. 21, Issue 9, 2017, pp. 1-19.
[29]. C.-M. Tîlmaciu, M. C. Morris, Carbon nanotube biosensors, *Front. Chem.*, Vol. 3, 2015, pp. 1-21.
[30]. A. Dasgupta, L. P. Rajukumar, C. Rotella, Y. Lei, M. Terrones, Covalent three-dimensional networks of graphene and carbon nanotubes: Synthesis and environmental applications, *Nano Today*, Vol. 12, 2017, pp. 116-135.
[31]. Z. Zhu, An overview of carbon nanotubes and graphene for biosensing applications, *Nano-Micro Lett.*, Vol. 9, Issue 3, 2017, pp. 1-24.
[32]. J. Seo, *et al.*, Immunosensor employing stable, Solid 1-amino-2-naphthyl phosphate and ammonia-borane toward ultrasensitive and simple point-of-care testing, *ACS Sensors*, Vol. 2, Issue 8, 2017, pp. 1240-1246.

[33]. A. C. M. S. Dias, S. L. R. Gomes-Filho, M. M. S. Silva, R. F. Dutra, A sensor tip based on carbon nanotube-ink printed electrode for the dengue virus NS1 protein, *Biosens. Bioelectron.*, Vol. 44, Issue 1, 2013, pp. 216-221.
[34]. B. V. M. Silva, I. T. Cavalcanti, M. M. S. Silva, R. F. Dutra, A carbon nanotube screen-printed electrode for label-free detection of the human cardiac troponin T, *Talanta*, Vol. 117, 2013, pp. 431-437.
[35]. E. Sánchez-Tirado, C. Salvo, A. González-Cortés, P. Yáñez-Sedeño, F. Langa, J. M. Pingarrón, Electrochemical immunosensor for simultaneous determination of interleukin-1 beta and tumor necrosis factor alpha in serum and saliva using dual screen printed electrodes modified with functionalized double-walled carbon nanotubes, *Anal. Chim. Acta*, Vol. 959, 2017, pp. 66-73.
[36]. S. L. R. Gomes-Filho, A. C. M. S. Dias, M. M. S. Silva, B. V. M. Silva, R. F. Dutra, A carbon nanotube-based electrochemical immunosensor for cardiac troponin T, *Microchem. J.*, Vol. 109, 2013, pp. 10-15.
[37]. S. Ma, *et al.*, Interaction processes of ciprofloxacin with graphene oxide and reduced graphene oxide in the presence of montmorillonite in simulated gastrointestinal fluids, *Sci. Rep.*, Vol. 7, Issue 1, 2017, pp. 1-11.
[38]. R. Raccichini, A. Varzi, S. Passerini, B. Scrosati, The role of graphene for electrochemical energy storage, *Nat. Mater.*, Vol. 14, Issue 3, 2015, pp. 271-279.
[39]. H. Sadegh, Development of graphene oxide from graphite: A review on synthesis, characterization and its application in wastewater treatment, *Rev. Adv. Mater. Sci.*, Vol. 49, 2017, pp. 38-43.
[40]. L. G. Guex, *et al.*, Experimental review: Chemical reduction of graphene oxide (GO) to reduced graphene oxide (rGO) by aqueous chemistry, *Nanoscale*, Vol. 9, Issue 27, 2017, pp. 9562-9571.
[41]. J. Yukird, T. Wongtangprasert, R. Rangkupan, O. Chailapakul, T. Pisitkun, N. Rodthongkum, Label-free immunosensor based on graphene/polyaniline nanocomposite for neutrophil gelatinase-associated lipocalin detection, *Biosens. Bioelectron.*, Vol. 87, 2017, pp. 249-255.
[42]. P. K. Drain, *et al.*, Diagnostic point-of-care tests in resource-limited settings., *Lancet. Infect. Dis.*, Vol. 14, Issue 3, March 2014, pp. 239-249.
[43]. B. V. M. Silva, B. A. G. Rodríguez, G. F. Sales, M. D. P. T. Sotomayor, R. F. Dutra, An ultrasensitive human cardiac troponin T graphene screen-printed electrode based on electropolymerized-molecularly imprinted conducting polymer, *Biosens. Bioelectron.*, Vol. 77, 2016, pp. 978-985.
[44]. S. X. Lee, H. N. Lim, I. Ibrahim, A. Jamil, A. Pandikumar, N. M. Huang, Horseradish peroxidase-labeled silver/reduced graphene oxide thin film-modified screen-printed electrode for detection of carcinoembryonic antigen, *Biosens. Bioelectron.*, Vol. 89, 2017, pp. 673-680.
[45]. G. Doria, *et al.*, Noble metal nanoparticles for biosensing applications, *Sensors*, Vol. 12, Issue 12, Feb. 2012, pp. 1657-1687.
[46]. Y. Li, H. J. Schluesener, S. Xu, Gold nanoparticle-based biosensors, *Gold Bull.*, Vol. 43, Issue 1, March 2010, pp. 29-41.
[47]. H. Malekzad, P. Sahandi Zangabad, H. Mirshekari, M. Karimi, M. R. Hamblin, Noble metal nanoparticles in biosensors: recent studies and applications, *Nanotechnol. Rev.*, Vol. 6, Issue 3, January 2017, pp. 301-329.
[48]. M. Holzinger, A. Le Goff, S. Cosnier, Nanomaterials for biosensing applications: A review, *Front. Chem.*, Vol. 2, 2014, 63.
[49]. N. Li, P. Zhao, D. Astruc, Anisotropic gold nanoparticles: synthesis, properties, applications, and toxicity, *Angew. Chemie Int. Ed.*, Vol. 53, Issue 7, February 2014, pp. 1756-1789.
[50]. F. Inci, *et al.*, Multitarget, quantitative nanoplasmonic electrical field-enhanced resonating device (NE ^{2} RD) for diagnostics, *Proceedings of Natl. Acad. Sci.*, Vol. 112, Issue 32, August 2015, pp. E4354-E4363.
[51]. S. Kumar, W. Ahlawat, R. Kumar, N. Dilbaghi, Graphene, carbon nanotubes, zinc oxide and gold as elite nanomaterials for fabrication of biosensors for healthcare, *Biosens. Bioelectron.*, Vol. 70, August 2015, pp. 498-503.
[52]. M. Jacobs, A. Panneer Selvam, J. E. Craven, S. Prasad, Antibody-conjugated gold nanoparticle-based immunosensor for ultra-sensitive detection of troponin-T, *J. Lab. Autom.*, Vol. 19, Issue 6, Dec. 2014, pp. 546-554.
[53]. S. Sabouri, H. Ghourchian, M. Shourian, M. Boutorabi, A gold nanoparticle-based immunosensor for the chemiluminescence detection of the hepatitis B surface antigen, *Anal. Methods*, Vol. 6, Issue 14, June 2014, pp. 5059-5066.

[54]. R. El-Dessouky, M. Georges, H. M. E. Azzazy, Silver nanostructures: Properties, synthesis, and biosensor applications, *ACS Symposium Series*, Vol. 1112, 2012, pp. 359-404.
[55]. X. Lu, M. Rycenga, S. E. Skrabalak, B. Wiley, Y. Xia, Chemical synthesis of novel plasmonic nanoparticles, *Annu. Rev. Phys. Chem.*, Vol. 60, 2009, pp. 167-192.
[56]. Z. Farka, T. Juřík, D. Kovář, L. Trnková, P. Skládal, Nanoparticle-based immunochemical biosensors and assays: Recent advances and challenges, *Chem. Rev.*, Vol. 117, Issue 15, August 2017, pp. 9973-10042.
[57]. R. El-Dessouky, M. Georges, H. M. E. Azzazy, Silver nanostructures: Properties, synthesis, and biosensor applications, *ACS Symposium Series*, Vol. 1112, 2012, pp. 359-404.
[58]. N. Hao, *et al.*, An electrochemical immunosensing method based on silver nanoparticles, *J. Electroanal. Chem.*, Vol. 656, Issues 1-2, 2011, pp. 50-54.
[59]. S. Felici, *et al.*, Towards a model of electrochemical immunosensor using silver nanoparticles, *Procedia Technol.*, Vol. 27, January 2017, pp. 155-156.
[60]. X. Che, R. Yuan, Y. Chai, J. Li, Z. Song, J. Wang, Amperometric immunosensor for the determination of α-1-fetoprotein based on multiwalled carbon nanotube-silver nanoparticle composite, *J. Colloid Interface Sci.*, Vol. 345, Issue 2, May 2010, pp. 174-180.
[61]. Z. H. Ibupoto, N. Jamal, K. Khun, X. Liu, M. Willander, A potentiometric immunosensor based on silver nanoparticles decorated ZnO nanotubes, for the selective detection of d-dimer, *Sensors Actuators B Chem.*, Vol. 182, June 2013, pp. 104-111.
[62]. J. B. Haun, T.-J. Yoon, H. Lee, R. Weissleder, Magnetic nanoparticle biosensors, *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology*, Vol. 2, Issue 3, May 2010, pp. 291-304.
[63]. J. B. Haun, T.-J. Yoon, H. Lee, R. Weissleder, Magnetic nanoparticle biosensors, *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology*, Vol. 2, Issue 3, May 2010, pp. 291-304.
[64]. L. Syedmoradi, M. Daneshpour, M. Alvandipour, F. A. Gomez, H. Hajghassem, K. Omidfar, Point of care testing: The impact of nanotechnology, *Biosens. Bioelectron.*, Vol. 87, January 2017, pp. 373-387.
[65]. M. Martín, *et al.*, Rapid Legionella pneumophila determination based on a disposable core-shell Fe_3O_4 @poly(dopamine) magnetic nanoparticles immunoplatform, *Anal. Chim. Acta*, Vol. 887, August 2015, pp. 51-58.
[66]. V. Singh, S. Krishnan, Voltammetric Immunosensor Assembled on carbon-pyrenyl nanostructures for clinical diagnosis of type of diabetes, *Anal. Chem.*, Vol. 87, Issue 5, March 2015, pp. 2648-2654.
[67]. Y. Zhang, T. R. Nayak, H. Hong, W. Cai, Biomedical applications of zinc oxide nanomaterials., *Curr. Mol. Med.*, Vol. 13, Issue 10, December 2013, pp. 1633-1645.
[68]. S. Kumar, W. Ahlawat, R. Kumar, N. Dilbaghi, Graphene, carbon nanotubes, zinc oxide and gold as elite nanomaterials for fabrication of biosensors for healthcare, *Biosens. Bioelectron.*, Vol. 70, August 2015, pp. 498-503.
[69]. M. Marie, S. Mandal, O. Manasreh, An electrochemical glucose sensor based on zinc oxide nanorods, *Sensors*, Vol. 15, Issue 12, July 2015, pp. 18714-18723.
[70]. M. A. Seia, S. V. Pereira, M. A. Fernández-Baldo, I. E. De Vito, J. Raba, G. A. Messina, Zinc oxide nanoparticles based microfluidic immunosensor applied in congenital hypothyroidism screening, *Anal. Bioanal. Chem.*, Vol. 406, Issue 19, July 2014, pp. 4677-4684.
[71]. A. T. Giannitsis, Microfabrication of biomedical lab-on-chip devices. A review, *Est. J. Eng.*, Vol. 17, Issue 2, 2011, pp. 109-139.
[72]. Y. Temiz, R. D. Lovchik, G. V. Kaigala, E. Delamarche, Lab-on-a-chip devices: How to close and plug the lab?, *Microelectron. Eng.*, Vol. 132, January 2015, pp. 156-175.
[73]. M. Zarei, Advances in point-of-care technologies for molecular diagnostics, *Biosens. Bioelectron.*, Vol. 98, December 2017, pp. 494-506.
[74]. P. K. Drain, *et al.*, Diagnostic point-of-care tests in resource-limited settings., *Lancet. Infect. Dis.*, Vol. 14, Issue 3, March 2014, pp. 239-249.
[75]. F. A. Gomez, Paper microfluidics in bioanalysis, *Bioanalysis*, Vol. 6, Issue 21, November 2014, pp. 2911-2914.
[76]. R. Amin, *et al.*, 3D-printed microfluidic devices, *Biofabrication*, Vol. 8, Issue 2, June 2016, 22001.
[77]. J. Hu, *et al.*, Advances in paper-based point-of-care diagnostics, *Biosens. Bioelectron.*, Vol. 54, April 2014, pp. 585-597.

[78]. C. M. B. Ho, S. H. Ng, K. H. H. Li, Y.-J. Yoon, 3D printed microfluidics for biological applications., *Lab Chip*, Vol. 15, Issue 18, 2015, pp. 3627-3637.
[79]. K. Yamada, T. G. Henares, K. Suzuki, D. Citterio, Paper-based inkjet-printed microfluidic analytical devices, *Angew. Chemie Int. Ed.*, Vol. 54, Issue 18, April 2015, pp. 5294-5310.
[80]. R. Amin, *et al.*, 3D-printed microfluidic devices, *Biofabrication*, Vol. 8, Issue 2, Jun. 2016, 22001.
[81]. J. Hu, *et al.*, Advances in paper-based point-of-care diagnostics, *Biosens. Bioelectron.*, Vol. 54, April 2014, pp. 585-597.
[82]. M. Zarei, Advances in point-of-care technologies for molecular diagnostics, *Biosens. Bioelectron.*, Vol. 98, December 2017, pp. 494-506.
[83]. O. Strohmeier, *et al.*, Centrifugal microfluidic platforms: Advanced unit operations and applications, *Chem. Soc. Rev.*, Vol. 44, Issue 17, August 2015, pp. 6187-6229.
[84]. R. Burger, L. Amato, A. Boisen, Detection methods for centrifugal microfluidic platforms, *Biosens. Bioelectron.*, Vol. 76, February 2016, pp. 54-67.

ANEXO A - NORMAS PARA SUBMISSÃO AO PERIÓDICO TALANTA

GUIDE FOR AUTHORS

PREPARATION

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns.

The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - unnumbered sections

Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross referencing text: refer to the subsection by heading as opposed to simply 'the text'.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results

Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information
• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lowercase superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract
A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract
A graphical abstract is mandatory for this journal. It should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site. Authors can make use of Elsevier's Illustration Services to ensure the best presentation of their images also in accordance with all technical requirements.

Highlights
Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). You can view example Highlights on our information site.

Keywords
Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations
Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).
Formatting of funding sources
List funding sources in this standard way to facilitate compliance to funder's requirements: Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].
It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding. If no funding has been provided for the research, please include the following sentence: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Nomenclature
All nomenclature, abbreviation, and units should conform as closely as possible to the rules established by IUPAC (available online at http://www.iupac.org/.)

Math formulae
Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise,

please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or
use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below): EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF, or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure it self) and a description of

the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

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Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

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Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

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This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

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Our policy now requires listing of the title of a cited article. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
Example: '..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ....'
List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.

Examples:

Reference to a journal publication:
[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59.

Reference to a book:
[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.

Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.

Reference to a website:
[4] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).

Reference to a dataset:
[dataset] [5] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/ xwj98nb39r.1.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 150 MB in total. Any single file should not exceed 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including Science Direct. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

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Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

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This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project. Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking
If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on Science Direct with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described. There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.
For supported data repositories a repository banner will automatically appear next to your published article on Science Direct.
In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

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This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. Before submitting your article, you can deposit the relevant datasets to *Mendeley Data*. Please include the DOI of the deposited dataset(s) in your main manuscript file. The datasets will be listed and directly accessible to readers next to your published article online.
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Data in Brief
You have the option of converting any or all parts of your supplementary or additional raw data into one or multiple data articles, a new kind of article that houses and describes your data. Data articles ensure that your data is actively reviewed, curated, formatted, indexed, given a DOI and publicly available to all upon publication. You are encouraged to submit your article for *Data in Brief* as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to *Data in Brief* where it will be editorially reviewed and published in the open access data journal, *Data in Brief*. Please note an open access fee of 500 USD is payable for publication in *Data in Brief*. Full details can be found on the Data in Brief website. Please use this template to write your Data in Brief. *Methods X*
You have the option of converting relevant protocols and methods into one or multiple Methods X articles, a new kind of article that describes the details of customized research methods. Many researchers spend a significant amount of time on developing methods to fit their specific needs or setting, but often without getting credit for this part of their work. Methods X, an open access journal, now publishes this information in order to make it searchable, peer reviewed, citable and reproducible. Authors are encouraged to submit their Methods X article as an additional item directly alongside the revised version of their manuscript. If your research article is accepted, your methods article will automatically be transferred over to MethodsX where it will be editorially reviewed. Please note an open access fee is payable for publication in Methods X. Full details can be found on the Methods X website. Please use this template to prepare your MethodsX article.

Data statement
To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why

during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

AudioSlides

The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available. Authors of this journal will automatically receive an invitation e-mail to create an Audio Slides presentation after acceptance of their paper.

Interactive plots

This journal enables you to show an Interactive Plot with your article by simply submitting a data file. Full instructions.

ANEXO B - NORMAS PARA SUBMISSÃO AO PERIÓDICO ANALYTICA CHIMICA ACTA

GUIDE FOR AUTHORS

PREPARATION

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More
information on types of peer review.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Materials and methods

The experimental methods should be described after the introductory material. Detailed experimental descriptions should be restricted to one section of the paper. Sufficient detail should be given to allow any experienced worker to implement the procedures described. Procedural steps should not be numbered.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

This should include key findings of the research, quantitative analytical performance figures (if appropriate) and their significance to real sample matrices.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information at the beginning of the manuscript file.

• ***Article title.*** Concise and informative. Titles are often used in information-retrieval systems. No abbreviations should be used and avoid formulae where possible.
• ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). No abbreviations should be used. These keywords will be used for indexing purposes.

Abbreviations

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Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:
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It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding. If no funding has been provided for the research, please include the following sentence:

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Please use L for litres. Always leave a space between units and use superscripts rather than /. For instance: use **mg mL-1** and not **mg/ml**. Do not use ppm or ppb to denote solid/liquid concentrations. Do not use abbreviations in the title or keywords. Define abbreviations that are not standard in this field at their first occurrence in both the abstract and the main text. Ensure consistency of abbreviations throughout the remainder of the manuscript. In all other cases, the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) should be followed, and attention should be given to the recommendations of the Analytical Chemistry Division in the journal *Pure and Applied Chemistry* (see also IUPAC Compendium of Analytical Nomenclature, Definitive Rules, 1987).

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the published version.
• Submit each illustration as a separate file.
A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.

Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
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Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
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ANEXO C - NORMAS PARA A SUBMISSÃO AO PERIÓDICO *BIOSENSORS & BIOELECTRONICS*

GUIDE FOR AUTHORS

Aims and Scope

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