UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOTECNOLOGIA
PÓS-GRADUAÇÃO EM BIOTECNOLOGIA

TALLITA STÉFANNE E SILVA

DESENVOLVIMENTO DE UM IMUNOSSENSOR IMPEDIMÉTRICO PARA A DETECÇÃO DO VÍRUS SINCICIAL RESPIRATÓRIO

PATOS DE MINAS – MG
JUNHO DE 2023

TALLITA STÉFANNE E SILVA

DESENVOLVIMENTO DE UM IMUNOSSENSOR IMPEDIMÉTRICO PARA A DETECÇÃO DO VÍRUS SINCICIAL RESPIRATÓRIO

Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Biotecnologia como requisito parcial para a obtenção do título de Mestre em Biotecnologia.

Orientador: Prof. Dr. Diego Leoni Franco

PATOS DE MINAS – MG

JUNHO DE 2023

Ficha Catalográfica Online do Sistema de Bibliotecas da UFU com dados informados pelo(a) próprio(a) autor(a).

S586 Silva, Tallita Stéfanne e, 1998-
2023 DESENVOLVIMENTO DE UM IMUNOSSENSOR IMPEDIMÉTRICO PARA A DETECÇÃO DO VÍRUS SINCICIAL RESPIRATÓRIO [recurso eletrônico] / Tallita Stéfanne e Silva. - 2023.

Orientador: Diego Leoni Franco.
Dissertação (Mestrado) - Universidade Federal de Uberlândia, Pós-graduação em Biotecnologia.
Modo de acesso: Internet.
Disponível em: http://doi.org/10.14393/ufu.di.2023.240 Inclui bibliografia.
Inclui ilustrações.

1. Biotecnologia. I. Franco, Diego Leoni ,1983-, (Orient.). II. Universidade Federal de Uberlândia. Pós- graduação em Biotecnologia. III. Título.

CDU: 60

Bibliotecários responsáveis pela estrutura de acordo com o AACR2:
Gizele Cristine Nunes do Couto - CRB6/2091
Nelson Marcos Ferreira - CRB6/3074

UNIVERSIDADE FEDERAL DE UBERLÂNDIA

Coordenação do Programa de Pós-Graduação em Biotecnologia
Av. Getúlio Vargas, 230, 3º andar, Sala 308 - Bairro Centro, Patos de Minas-MG, CEP 38700-128

Telefone: (34) 3823-3714 - Ramal 39 - www.ppgbiotec.ibtec.ufu.br - ppgbiotec@ibtec.ufu.br

ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Biotecnologia				
Defesa de:	Dissertação de Mestrado				
Data:	23/06/2023	Hora de início:	14h	Hora de encerramento:	18h
Matrícula do Discente:	42112BTC005				
Nome do Discente:	Tallita Stéfanne e Silva				
Título do Trabalho:	Desenvolvimento de um imunossensor impedimétrico para a detecção do vírus sincicial respiratório				
Área de concentração:	Biociências				
Linha de pesquisa:	Prospecção de proutos bioativos de interesse médico e industrial				
Projeto de Pesquisa de vinculação:	Desenvolvimento de um imunossensor impedimétrico para a detecção do vírus sincicial respiratório				

Reuniu-se por videoconferência, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação emBiotecnologia, assim composta: Professores Doutores: Lucas Franco Ferreira - ICT/UFVJM; Guilherme Ramos Oliveira e Freitas - IBTEC/UFU; e Diego Leoni Franco - IQ/UFU, orientador da candidata.

Iniciando os trabalhos o(a) presidente da mesa, Dr(a). Diego Leoni Franco, apresentou a Comissão Examinadora e a candidata, agradeceu a presença do público, e concedeu à Discente a palavra para a exposição do seu trabalho. A duração da apresentação da Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir a candidata. Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

Aprovada.

Esta defesa faz parte dos requisitos necessários à obtenção do título de Mestre.

O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

Documento assinado eletronicamente por **Diego Leoni Franco**, **Professor(a) do Magistério Superior**, em 23/06/2023, às 18:04, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Guilherme Ramos Oliveira e Freitas**, **Professor(a) do Magistério Superior**, em 23/06/2023, às 18:04, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Lucas Franco Ferreira**, **Usuário Externo**, em 23/06/2023, às 18:04, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **4590349** e o código CRC **C8D13884**.

Referência: Processo nº 23117.043677/2023-25

DESENVOLVIMENTO DE UM IMUNOSSENSOR IMPEDIMÉTRICO PARA A DETECÇÃO DO VÍRUS SINCICIAL RESPIRATÓRIO

Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Biotecnologia como requisito parcial para a obtenção do título de Mestre em Biotecnologia.

Aprovado em ___/___/___

BANCA EXAMINADORA

Prof. Dr. Diego Leoni Franco (Orientador)

Prof. Dr. Guilherme Ramos Oliveira e Freitas

Prof. Dr. Lucas Franco Ferreira

PATOS DE MINAS – MG

2023

Dedico esta dissertação a todos os meus professores: do ensino fundamental até o ensino superior.

Remember the moment
You left me alone and
Broke every promise you ever made
I was an ocean lost in the open
Nothing could take the pain away

So you can throw me to the wolves
Tomorrow I will come back
Leader of the whole pack
Beat me black and blue
Every wound will shape me
Every scar will build my throne

The sticks and the stones that
You used to throw have
Built me an empire, so don't even try
To cry me a river, 'cause I forgive you
You are the reason I still fight

So you can throw me to the wolves
Tomorrow I will come back
Leader of the whole pack
Beat me black and blue
Every wound will shape me
Every scar will build my throne

THRONE
BRING ME THE HORIZON

"*Somewhere, something incredible is waiting to be known.*"

– Carl Sagan

AGRADECIMENTOS

Ao **Prof. Dr. Diego Leoni Franco** por todo o auxílio durante a minha formação no ensino superior. Agradeço por ter aceitado me orientar da iniciação científica até o mestrado e por acreditar na minha capacidade como pesquisadora.

À minha família, especialmente minha mãe **Elaine Maria Vieira Silva**, meu pai **José dos Reis Silva** e minha avó **Marluce Rodrigues Vieira** por todo o amor e dedicação. Agradeço por todo o apoio e incentivo para que eu consiga seguir os meus sonhos.

À minha futura esposa **Júlia Duarte Megale** por sempre estar ao meu lado, mesmo nos momentos mais difíceis. Agradeço por toda a compreensão, carinho e reconhecimento ao longo de quase 4 anos juntas. Eu amo você!

Aos membros das minhas bancas ao longo da pós-graduação. Agradeço à **Profª. Drª. Claudilene Ribeiro Chaves**, ao **Prof. Dr. Guilherme Ramos Oliveira e Freitas** e ao **Prof. Dr. Lucas Franco Ferreira** por todas as críticas e sugestões para o enriquecimento deste trabalho.

Aos professores e técnicos da Universidade Federal de Uberlândia – Campus Patos de Minas por todos os ensinamentos e amparo durante minha formação. Especialmente agradeço à **Profª. Drª. Djenaine de Souza** e aos técnicos **Esp. Carla Ferreira de Lima**, **Drª. Luciana de Oliveira Almeida** e **Dr. Raoni Pais Siqueira**.

A todos os meus professores da educação básica pelo incentivo e estímulo durante o ensino fundamental e médio. Agradeço especialmente ao **Prof. Marcos Paulo da Silva** e ao **Prof. Sérgio Luis Silva** por despertarem minha paixão por ciências e matemática, disciplinas fundamentais durante toda a minha formação.

Aos colegas de laboratório que pude compartilhar todas as conquistas e frustrações durante meu tempo no LEABE. Especialmente aos meus amigos **Ana Hercília Magalhães Nunes Mapa**, **Lorranne Marins Gonçalves** e **Pedro Antônio Palhano Arantes Silva**, amizades que foram além da universidade e sempre terão um lugar especial no meu coração.

À **Lara Caetano da Silva** pela amizade e apoio durante toda a minha formação no ensino superior. Agradeço por sempre mostrar que sou capaz de conseguir o que almejo e por encorajar minha paixão pela ciência.

À **Esp. Gabriela Pereira e Sousa** pelo amparo psicológico durante toda a minha pós-graduação, muito importante para que eu chegasse até aqui.

Aos meus filhos felinos **Arya**, **Eclipse**, **François** e **Sr. Simpático** por serem meus ouvintes oficiais durante o preparo de apresentações orais. Agradeço pelo afeto e companhia durante toda a minha trajetória.

RESUMO

O vírus sincicial respiratório (RSV) é um importante patógeno respiratório que acomete principalmente crianças menores de cinco anos. Apesar de na maioria dos casos de infecção em pessoas adultas e imunocompetentes causar quadros leves sem complicações, em crianças e idosos é frequente o aparecimento de bonquiolite, bronquite e pneumonia. As formas de detecção do RSV incluem RT-qPCR, ensaio imunocromatográfico e ensaio de imunofluorescência indireto que apresentam desvantagens relacionadas ao tempo, preço e sensibilidade que podem ser contornadas pelo desenvolvimento de um imunossensor eletroquímico, um dispositivo de baixo custo, rápido, específico e de fácil manuseio. A ligação covalente é uma metodologia efetiva para a imobilização do anticorpo (Ab), de modo que a modificação eletroquímica do eletrodo de trabalho de carbono grafite de lapiseira (PGE), com polímeros condutores ou não, que apresentem grupos funcionais, são interessantes para esse procedimento. O ácido 3-aminofenilacético (3APA) apresenta as características desejadas e foi o monômero escolhido, com intuito de apresentar grupos carboxilas disponíveis após a formação polimérica. As técnicas de voltametria cíclica (CV) e de espectroscopia de impedância eletroquímica (EIS) foram usadas para a eletropolimerização e caracterização do material formado. Os CVs demonstraram que houve formação de poli(3APA) e 30 ciclos foram estabelecidos por apresentar a melhor resposta para o sistema. Estudos eletroquímicos e de pH em CV possibilitaram determinar o número de elétrons e a razão próton/elétron para apresentar a proposta do mecanismo de eletropolimerização do 3APA. O imunossensor foi desenvolvido sobre esta matriz com a ativação dos grupos carboxílicos do poli(3APA) em solução de 1-etil-3-(3-dimetilaminopropil)carbodiimida (EDC) / N-hidroxisuccinimida (NHS) seguido da reação de substituição nucleofílica com os grupos amino presentes no Ab. Após a imobilização de Ab, o PGE/poli(3APA) teve sua superfície bloqueada por glicina (Gly) para impedir interações inespecíficas, seguido da imobilização do antígeno (Ag). As detecções ocorreram por EIS e os dados foram avaliados pelos circuitos equivalentes. Houve o aumento nos valores de resistência à transferência de carga (Rtc) conforme o PGE foi modificado, na seguinte ordem: PGE < PGE/poli(3APA) < PGE/poli(3APA)/Ab < PGE/poli(3APA)/Ab/Ag. O sistema foi otimizado buscando as melhores condições para detecção. Foi usada a variação de resposta (ΔRtc) entre o sistema completo contendo Ab/Ag e o sistema contendo somente Ab (n = 3). Para o preparo do imunossensor foram escolhidos 100 ng de Ab, e o tempo de imobilização de Ab e Gly foram de, respectivamente, 3 h e 50 min. O tempo de resposta do sistema, com a imobilização de Ag foi de 30 min. Com a curva de calibração do sistema otimizado, o LOD calculado foi de 27,65 $PFU.mL^{-1}$ e o LOQ de 92,15 $PFU.mL^{-1}$, demonstrando que o imunossensor proposto é uma alternativa viável para a detecção de RSV.

Palavras-chave: Vírus sincicial respiratório; Imunossensor eletroquímico; Ácido 3-aminofenilacético; Eletropolimerização; Voltametria cíclica; Espectroscopia de impedância eletroquímica.

ABSTRACT

Respiratory syncytial virus (RSV) is an important respiratory pathogen that mainly affects children under the age of five. While RSV infections in healthy, immunocompetent adults usually result in mild uncomplicated cases, infants and the elderly often experience bronchiolitis, bronchitis, and pneumonia. Current detection methods for RSV include RT-qPCR, immunochromatography, and indirect immunofluorescence assays, but they have disadvantages related to time, cost, and sensitivity. These limitations can be overcome by developing an electrochemical immunosensor, a low-cost, rapid, specific, and user-friendly device. Covalent binding is an effective methodology for antibody (Ab) immobilization, and the electrochemical modification of a graphite pencil lead working electrode (PGE) with conductive or non-conductive polymers that possess functional groups is of interest for this procedure. 3-aminophenylacetic acid (3APA) exhibits the desired characteristics and was chosen as the monomer to provide available carboxyl groups after polymerization. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques were employed for electropolymerization and characterization of the formed material. The CV results demonstrated the formation of poly(3APA), with 30 cycles established as the optimal condition due to the best response observed in the system. Electrochemical and pH studies in CV allowed for the determination of the number of electrons and the proton-to-electron ratio, providing insight into the proposed mechanism of 3APA electropolymerization. The immunosensor was developed on this matrix by activating the carboxylic groups of poly(3APA) in a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) / N-hydroxysuccinimide (NHS), followed by nucleophilic substitution reaction with the amino groups present in the antibody (Ab). After Ab immobilization, the PGE/poly(3APA) surface was blocked by glycine (Gly) to prevent non-specific interactions, followed by antigen (Ag) immobilization. Detection was performed using EIS, and the data were evaluated using equivalent circuits. The values of charge transfer resistance (Rtc) increased as the PGE was modified in the following order: PGE < PGE/poly(3APA) < PGE/poly(3APA)/Ab < PGE/poly(3APA)/Ab/Ag. The system was optimized to achieve the best detection conditions. The response variation (ΔRtc) between the complete system containing Ab/Ag and the system containing only Ab (n = 3) was utilized. For the preparation of the immunosensor, 100 ng of Ab was chosen, and the immobilization times for Ab and Gly were, respectively, 3 h and 50 min. The response time of the system, with Ag immobilization, was 30 min. Using the calibration curve of the optimized system, the calculated limit of detection (LOD) was 27.65 $PFU.mL^{-1}$, and the limit of quantification (LOQ) was 92.15 $PFU.mL^{-1}$, demonstrating that the proposed immunosensor is a viable alternative for the detection of RSV.

Keywords: *Respiratory syncytial virus; Electrochemical immunosensor; 3-aminophenylacetic acid; Electropolymerization; Cyclic voltammetry; Electrochemical impedance spectroscopy.*

LISTA DE ABREVIATURAS E SIGLAS

3APA	Ácido 3-aminofenilacético
Ab	Anticorpo
Ag	Antígeno
cDNA	DNA complementar
CV	Voltametria cíclica
DNA	Ácido desoxirribonucleico
EDC	1-etil-3-(3-dimetilaminopropil)carbodiimida
EIS	Espectroscopia de impedância eletroquímica
F	Glicoproteína de fusão
Gly	Glicina
IgG	Imunoglobulina G
IRA	Infecção respiratória aguda
IRATRI	Infecção respiratória aguda no trato respiratório inferior
IRATRS	Infecção respiratória aguda no trato respiratório superior
L	RNA polimerase dependente de RNA viral
LOD	Limite de detecção
LOQ	Limite de quantificação
M	Matriz
mRNA	RNA mensageiro
N	Nucleoproteína
NHS	N-hidroxisuccinimida
P	Fosfoproteína
PCR	*Polymerase chain reaction*
qPCR	*Real time* PCR
RNA	Ácido ribonucleico
RSV	Vírus sincicial respiratório
RT-qPCR	*Reverse transcription* qPCR
Rtc	Resistência a transferência de carga
SH	Gliproteína hidrofóbica pequena

LISTA DE SÍMBOLOS E UNIDADES

β	Beta
π	Pi
σ	Sigma
%	Por cento
g	Grama
h	Hora
min	Minutos
mL	Mililitro
ng	Nanogramas
PFU	Unidade formadora de placa

LISTA DE FIGURAS

SUMÁRIO

CAPÍTULO 1: REFERENCIAL TEÓRICO

1 INTRODUÇÃO

O vírus sincicial respiratório (RSV) é um patógeno responsável por infecções nas vias respiratórias, majoritariamente em crianças menores de cinco anos. Em casos graves, há infecção respiratória aguda no trato respiratório inferior (IRATRI), podendo ocasionar em pneumonia e bronquiolite, doenças presentes entre as principais causas de morbidade e mortalidade em pacientes com menos de 5 anos (BORCHERS et al., 2013; LIU et al., 2016; SHI et al., 2017). No geral, o diagnóstico é feito por avaliação clínica, mas devido ao elevado número de microrganismos responsáveis por ocasionar os mesmos quadros do RSV, exames de diagnóstico laboratorial podem ser necessários (JHA et al., 2016; RALSTON et al., 2017). O ensaio de imunofluorescência indireto é a principal técnica para detecção do vírus, com boa sensibilidade e baixo custo, mas apresenta menor agilidade em sua execução. Outra alternativa á a *reverse transcription real time quantitative polymerase chain reaction* (RT-qPCR), vantajosa devido à alta sensibilidade. Porém, é uma técnica cara em relação a equipamentos e reagentes e necessita de profissionais qualificados. Também existem testes imunocromatográficos para detecção de antigenos, conhecidos popularmente como testes rápidos, que são baratos e específicos, mas com sensibilidade de apenas 75.3% durante surtos do vírus (BRUNING et al., 2017; JAIN; SCHWEITZER; JUSTICE, 2022). Desse modo, é necessário o desenvolvimento de uma forma de detecção que una as vantagens dos testes já existentes, ou seja, uma técnica rápida, sensível, específica e de baixo custo.

Biossensores são capazes de detectar analitos específicos através da conversão de um sinal bioquímico, proveniente de um componente biológico imobilizado, para um sinal detectável por meio de um transdutor (SCHULTZ; TAYLOR, 1996), que pode ser óptico (VÉDRINE et al., 2003), eletroquímico (STÉFANNE E SILVA et al., 2020), ou piezoelétrico (ZHOU et al., 2002), entre os mais comuns. Além disso, podem ser imobilizadas diferentes tipos de moléculas biológicas, como enzimas (STÉFANNE E SILVA et al., 2020), anticorpos (VENTURINI ULIANA; YAMANAKA, 2019), ácidos nucleicos (DA FONSECA ALVES et al., 2019) e até mesmo células inteiras (VÉDRINE et al., 2003). A aplicação de biossensores se estende por uma variedade de áreas (MEHROTRA, 2016), como a ambiental (STÉFANNE E SILVA et al., 2020; VÉDRINE et al., 2003; VENTURINI ULIANA; YAMANAKA, 2019), a alimentícia (SOARES et al., 2019) e a da saúde (DA FONSECA ALVES et al., 2019; RAMANATHAN; JÖNSSON; DANIELSSON, 2001; ZHOU et al., 2002).

Os anticorpos, componentes biológicos que atuam no sistema imunológico (ABBAS; LITCHTMAN; PILLAI, 2019a), podem ser imobilizados em um transdutor para o desenvolvimento de imunossensores. A ligação antígeno-anticorpo é específica e a sensibilidade do dispositivo dependerá da eficiência da imobilização, da estabilidade da biomolécula imobilizada da afinidade entre essa reação, em que ligações irreversíveis são vantajosas, pois garantem alta sensibilidade (GIZELI; LOWE, 1996; HOCK, 1997). Sendo assim, o desenvolvimento de um imunossensor é interessante para diagnóstico de RSV.

Entre os transdutores, o eletroquímico apresenta como vantagens a miniaturização e a sensibilidade. Nesse tipo de dispositivo, um eletrodo de trabalho faz a conversão do sinal bioquímico em um sinal elétrico quantificável, possível devido ao fluxo de elétrons presentes na solução a ser analisada, em que o valor adquirido é proporcional à concentração do analito (ABDULBARI; BASHEER, 2017; WANG, 1996, 2000). Eletrodos a base de carbono são comumente usados no desenvolvimento de biossensores. Eles podem ser carbono vítreo (RAMANATHAN; JÖNSSON; DANIELSSON, 2001), pasta de carbono (ZHANG et al., 2007), nanotubos de carbono (WANG et al., 2003), carbono de grafite de lapiseira (STÉFANNE E SILVA et al., 2020), entre outros. O eletrodo de carbono grafite de lapiseira (PGE) tem mostrado resultados tão satisfatórios quanto os demais eletrodos a base de carbono, além de ser uma alternativa de baixo custo, de fácil acesso e de fácil manuseio (ALVES et al., 2017; DA FONSECA ALVES et al., 2019; SOARES et al., 2019; STÉFANNE E SILVA et al., 2020; TORRINHA et al., 2018).

Para o desenvolvimento de biossensor, é necessária uma forma eficiente de imobilização do componente biológico sobre o transdutor. Os tipos de imobilização mais comuns são ligação covalente, aprisionamento, *cross-linking* e adsorção física (TAYLOR, 1996). No método de ligação covalente, o eletrodo de trabalho pode ter sua superfície modificada com um filme polimérico, condutor ou não, geralmente formado por voltametria cíclica (CV), técnica eletroquímica que oferece informações qualitativas sobre a modificação. O principal objetivo dessa modificação é obter um polímero com grupos funcionais disponíveis para a ligação com o componente biológico (BATFLETT, 1996; PACHECO et al., 2013). Monômeros derivados da anilina, como o ácido 3-aminofenilacético (3APA), são bons candidatos para eletropolimerização na superfície do eletrodo devido ao possível caráter condutor desses materiais, o que melhora a resposta de detecção do analito (TAHIR; ALOCILJA; GROOMS, 2005).

Desta forma, este trabalho possui dois grandes objetivos: a modificação de PGEs com polímeros derivados do 3APA, seguido da caracterização eletroquímica do material formado e

utilização desta plataforma modificada para o desenvolvimento de um imunossensor impedimétrico para o RSV. Até onde temos conhecimento, não há na literatura um biossensor eletroquímico baseado em PGEs modificados com polímero derivado de 3APA para esta aplicação.

2 REFERENCIAL TEÓRICO

2.1 Infecção respiratória aguda (IRA)

Infecções respiratórias agudas (IRAs) são caracterizadas pela obstrução da passagem de ar no sistema respiratório e é uma das principais causas de hospitalização de crianças em todo o mundo (BERMAN, 1991). Elas podem ser classificadas como infecção respiratória aguda no trato respiratório superior (IRATRS) e infecção respiratória aguda no trato respiratório inferior (IRATRI). A IRATRS atinge desde as vias aéreas das narinas até as cordas vocais na laringe, e é a doença mais frequente em humanos e a mais comum na infância, já que crianças com até 5 anos desenvolvem, em média, de 6 a 8 infecções por ano (MONTO, 1994; SIMOES et al., 2006). Apesar de não gerar casos graves, IRATRS podem causar epidemias generalizadas que permitem a circulação constante de patógenos (YEN et al., 2019).

A IRATRI atinge a traqueia, brônquios, bronquíolos ou alvéolos, ocasionando em quadros de bronquiolite, bronquite e pneumonia (BASILIO et al., 2017; SIMOES et al., 2006). Por desenvolver casos graves, as IRATRIs requerem bastante atenção, principalmente por serem a maior causa infecciosa de morte em infectados com até 5 anos de idade (YEN et al., 2019). Dados da OMS de 2000 a 2003 demonstram que 19% das mortes de crianças dessa faixa etária foram causadas por pneumonia (BRYCE et al., 2005). Os três principais causadores da doença são as bactérias *Haemophilus influenzae* tipo b e *Streptococcus pneumoniae* (imunopreviníveis) e o vírus sincicial respiratório (ainda sem imunoprevenção para essa faixa etária) (HARRIS, 2023; NAIR *et al.*, 2010).

2.2 Vírus sincicial respiratório (RSV)

O RSV, pertencente ao gênero *Orthopneumovirus* e família *Pneumoviridae* (ICTV, 2021), foi isolado pela primeira vez de chimpanzés em 1956 (MORRIS; BLOUNT; SAVAGE, 1956) e posteriormente, em 1957, de bebês (CHANOCK; ROIZMAN; MYERS, 1957). O vírus é um patógeno humano comum, caracterizado por não desenvolver imunidade a longo prazo,

acarretando em reinfecções ao longo da vida. O primeiro contágio, em 90% dos casos, acontece até os 2 anos de idade (JAIN; SCHWEITZER; JUSTICE, 2022). Comumente, seu quadro clínico é caracterizado por IRATRS, sem grandes complicações, mas, em menores proporções, pode causar IRATRI em crianças, idosos e adultos imunocomprometidos. Devido a isso, o vírus se tornou um patógeno importante, podendo gerar surtos em todo o mundo, principalmente em países subdesenvolvidos (JHA et al., 2016; SHI et al., 2017). O RSV se apresenta em dois subgrupos antigênicos (A e B) que normalmente circulam juntos, mas com predominância de um subtipo sobre o outro. (MUFSON et al., 1985).

2.2.1 Epidemiologia

Aproximadamente 30 milhões de casos de RSV acontecem anualmente, acarretando em mais de 100 mil mortes de crianças. Os países subdesenvolvidos apresentam alta taxa de mortalidade pelo vírus, devido ao diagnóstico tardio, correspondendo a 90% dos óbitos pela infecção viral (PATH, 2017). Em pacientes com menos de 5 anos, é comum que o patógeno cause bronquiolite ou pneumonia devido a IRATRI (JAIN; SCHWEITZER; JUSTICE, 2022). Dentre as internações e as mortes hospitalares causadas pelo vírus, 45% correspondem a crianças com menos de 6 meses (SHI et al., 2017).

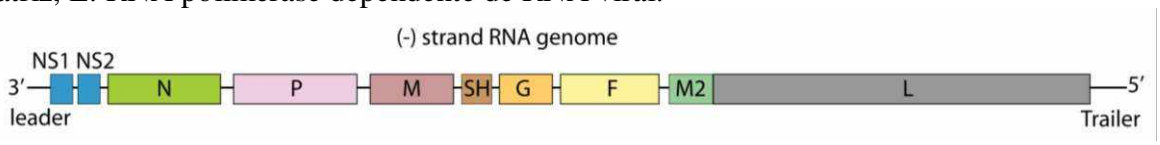
No Brasil, estudos realizados em um hospital de São Paulo – São Paulo entre 2009 e 2013, demonstraram que 14% das crianças infectadas com RSV apresentaram quadro mais grave e precisaram ser internadas. A idade média desse grupo era de 1,6 anos, enquanto crianças infectadas, mas sem complicações tinham idade média de 3,1 anos, demonstrando que pacientes mais novos são mais vulneráveis à infecção (CRUZ et al., 2021). Um estudo realizado em pacientes de até 5 anos em um hospital de Uberlândia – Minas Gerais entre 2010 e 2015 demonstrou que a detecção de RSV em crianças hospitalizadas ocorreu em uma frequência 2,3 vezes maior do que outros vírus respiratórios (rhinovirus, influenzavirus, parainfluenzavirus, adenovirus e metapneumovirus), demonstrando uma maior gravidade do patógeno e reafirmando a sua importância (COSTA et al., 2022)

2.2.2 Estrutura e replicação

O RSV é um vírus envelopado de RNA fita simples, não segmentado, de sentido negativo pertencente a classe V do sistema de classificação de Baltimore. Seu genoma (**Figura 1**), com duas janelas de leitura aberta, é composto por 10 genes que codificam 11 proteínas

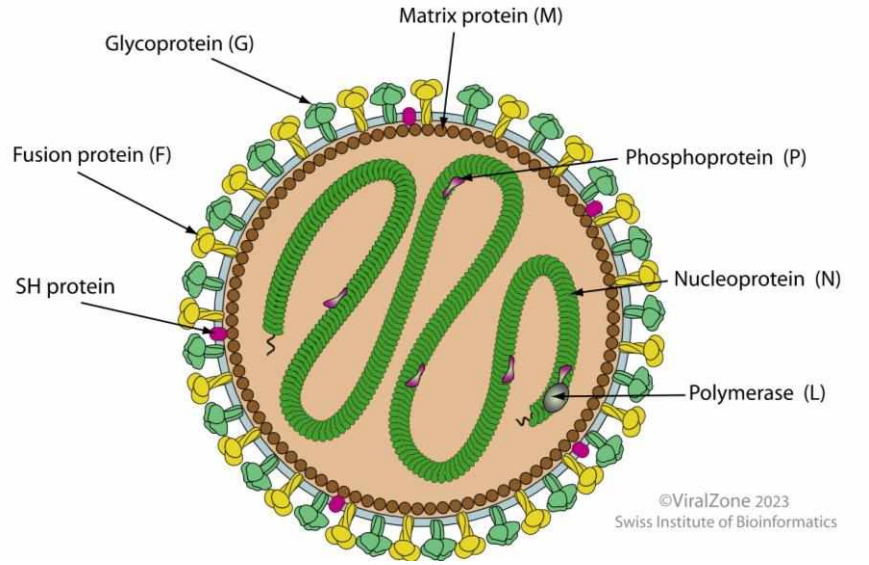
(BORCHERS et al., 2013). As partículas virais (**Figura 2**) são envoltas por um envelope lipídico que apresenta 3 glicoproteínas de superfície: a glicoproteína G, que garante a ligação do vírus à superfície da célula hospedeira; a glicoproteína de fusão (F), responsável pela fusão das membranas virais e celulares; e a glicoproteína hidrofóbica pequena (SH), presente em menor quantidade nas partículas virais, mas que é expressa em abundância na superfície das células infectadas. O núcleocapsídeo contém outras 6 proteínas estruturais: a RNA polimerase dependente de RNA viral (L), relacionada com várias atividades enzimáticas necessárias para a replicação; a nucleoproteína (N), que se liga fortemente ao RNA viral e evita sua degradação; a fosfoproteína (P), que impede a ligação entre a proteína N e os RNAs celulares; M2-1 e M2-2, codificadas a partir do gene M2, com duas janelas de leitura aberta e agem como reguladoras da transcrição e replicação viral; e a matriz (M), envolvida na coordenação da montagem das proteínas do envelope e do nucleocapsídeo, além de auxiliar na formação de novas partículas virais imaturas através da membrana celular hospedeira. Ademais, existem duas proteínas não estruturais: NS1 e NS2 (JHA et al., 2016; MELERO, 2007).

Figura 1 – Estrutura molecular do vírus sincicial respiratório humano. NS: proteínas não estruturais (NS1 e NS2), N: nucleoproteína, P: fosfoproteína, M: proteína de matriz, SH: glicoproteína hidrofóbica pequena, G: glicoproteína, F: proteína de fusão, M2: proteína de matriz, L: RNA polimerase dependente de RNA viral.



Fonte: ViralZone, 2023.

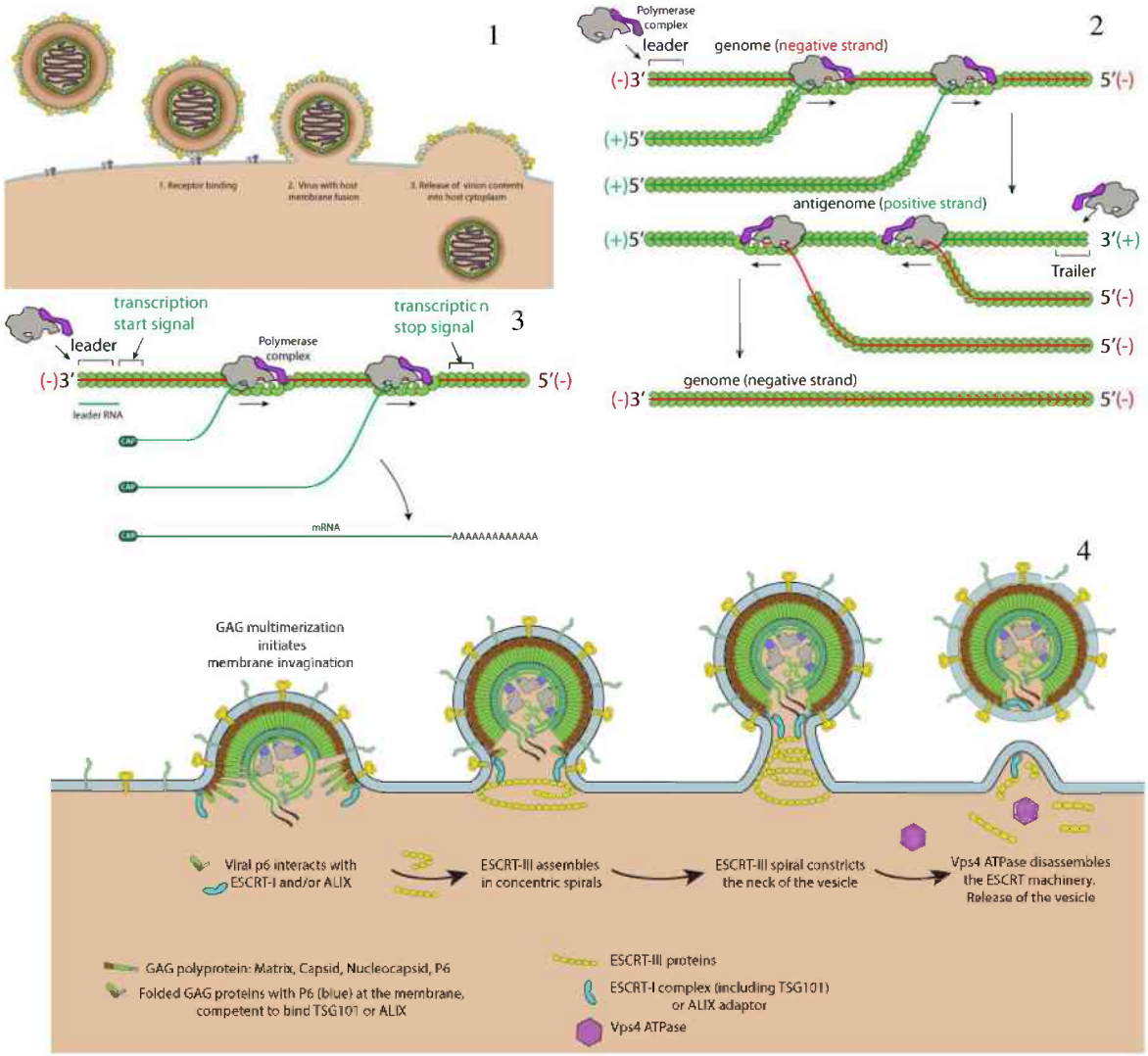
Figura 2 – Estrutura do vírion do vírus sincicial respiratório humano.



Fonte: ViralZone, 2023.

Quando o vírus entra em contato com a célula hospedeira, ocorre a ligação inicial entre a partícula viral e os receptores presentes na superfície celular pela glicoproteína G. Posteriormente, a proteína F é ativada para proporcionar a fusão, possibilitando a entrada do nucleocapsídeo viral no citoplasma da célula hospedeira. No meio intracelular, o RSV ativa a transcrição de seus genes de infecção por meio de um grupo de mRNAs que condiciona a síntese proteica aos ribossomos celulares. A replicação ocorre um pouco depois, gerando uma cópia completa de seu antigenoma (RNA viral de sentido positivo) que formará um complexo com a proteína N e servirá como molde para a síntese do genoma das novas partículas. As proteínas recém traduzidas migram para perto da membrana celular, onde ocorre a montagem dos novos vírus que, em seguida, serão liberados por brotamento (**Figura 3**) (COLLINS; FEARNS; GRAHAM, 2013; MELERO, 2007).

Figura 3 – Ciclo infeccioso do vírus sincicial respiratório humano. Fusão (1), transcrição sequencial (2), replicação (3) e liberação para o meio extracelular (4).



Fonte: ViralZone, 2023.

2.2.3 Fisiopatologia

A entrada do vírus no organismo do hospedeiro ocorre na mucosa do trato respiratório superior ou conjuntiva através do contato direto ou por gotículas contaminadas. O tempo de incubação é de 2-8 dias, variando de acordo com aspectos do hospedeiro, como idade ou quantidade de infecções anteriores. Em seguida, o vírus se espalha rapidamente, até chegar ao epitélio, onde seu crescimento é favorecido (JAIN; SCHWEITZER; JUSTICE, 2022; JHA et al., 2016).

Os mecanismos de imunidade inata são os primeiros que agem na defesa do hospedeiro, gerando uma resposta rápida, nas primeiras horas ou nos primeiros dias de infecção. Sendo assim, utiliza mecanismos já existentes no corpo, enquanto a resposta imune adaptativa está em desenvolvimento (ABBAS; LITCHTMAN; PILLAI, 2019b). Na infecção pelo RSV, a imunidade inata desempenha um importante papel na patogênese. As células epiteliais presentes nas vias aéreas, bem como os macrófagos alveolares e as células dendríticas, são responsáveis por ativar a imunidade inata e desenvolver a imunidade adaptativa contra o vírus. Essa etapa gera resposta inflamatória através da indução de citocinas e quimiocinas (TAYLOR, 2006).

A imunidade adaptativa não tem efeito imediato no organismo, pois é desenvolvida após o contato com o agente infeccioso. Ao contrário da imunidade inata, ela é específica e especializada, além de fortalecer seus mecanismos de defesa a cada novo contágio (ABBAS; LITCHTMAN; PILLAI, 2019b). A infecção por RSV não garante total proteção contra reinfecções, mas concede acúmulo de resistência à cada nova reinfecção do trato respiratório inferior, demonstrando que a imunidade é mais duradoura na infecção pulmonar do que no trato respiratório superior (TAYLOR, 2006). A resposta imunológica adaptativa ao vírus inclui a ativação de linfócitos humoral e citotóxico que, juntamente com a atividade citotóxica viral e a atividade citotóxica do hospedeiro, causam a necrose das células epiteliais do trato respiratório, ocasionando em uma pequena obstrução das vias aéreas por muco, e em casos mais graves, na obstrução dos alvéolos (JAIN; SCHWEITZER; JUSTICE, 2022).

2.2.4 Diagnóstico

O diagnóstico na maioria das infecções de RSV, sem complicações, é feito através da avaliação clínica dos sintomas do paciente. Porém, casos de IRATRI requerem maior cuidado devido a existência de bactérias causadoras da mesma infecção. Nessas circunstâncias, é preciso

confirmar que o RSV é o patógeno responsável pela infecção, afim de evitar a prescrição errônea de antibióticos (RALSTON et al., 2017). A detecção pode ser realizada quando o patógeno chega ao epitélio do hospedeiro, favorecendo o crescimento do vírus e aparição de sintomas no infectado (JAIN; SCHWEITZER; JUSTICE, 2022).

O ensaio de imunofluorescência indireto é o método mais comum no diagnóstico de RSV (TANG; LOH, 2014). Nele são usados dois anticorpos: o anticorpo primário, que garante a especificidade da reação; e um anti-anticorpo conjugado com fluorocromo que revela a reação (ABBAS; LITCHTMAN; PILLAI, 2019c). A técnica possui boa sensibilidade, é relativamente rápida (1-3 h) e tem baixo custo, porém seu rendimento é lento e necessita de mão de obra qualificada na operação, principalmente para avaliação dos resultados (MADELEY; PEIRIS, 2002).

Os ensaios imunocromatográficos, comumente conhecidos como testes rápidos, economizam tempo, com resultados prontos em 10-30 minutos, e são simples de serem manuseados, possibilitando o diagnóstico no próprio local e hora do atendimento ao paciente (JAIN; SCHWEITZER; JUSTICE, 2022). Estudos demonstram que os testes de primeira geração, baseados na mudança macroscópica de cor em contato com o antígeno, possuem sensibilidade de 67,8% e especificidade de 98,5% (RATH et al., 2012); enquanto os de segunda, que utilizam tecnologia avançada de fluxo lateral, marcadores fluorescentes e leitores automatizados, apresentam sensibilidade e especificidade de 75,3% e 98,7%, respectivamente (TUTTLE et al., 2015). Apesar do aumento da porcentagem da sensibilidade, é comum a aparição de falsos negativos, sendo essa a principal desvantagem dos ensaios imunocromatográficos (RALSTON et al., 2017).

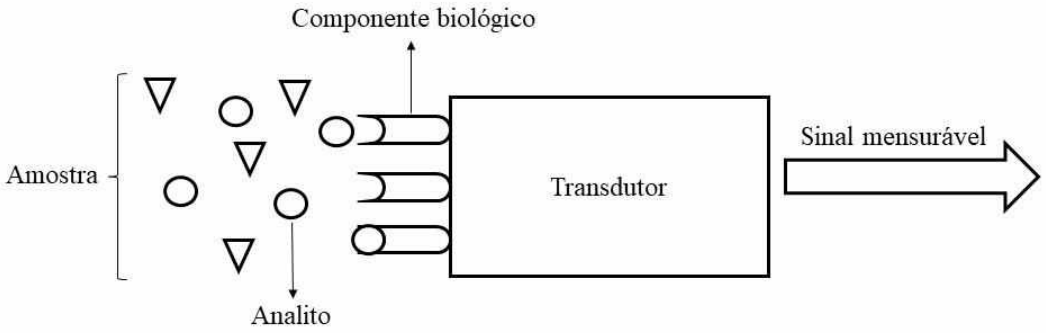
Em contrapartida, a PCR, descrita em 1980, é uma técnica de alta sensibilidade para a amplificação seletiva de fragmentos de ácidos nucleicos. O método consiste na replicação do fragmento de DNA de interesse por uma DNA polimerase termoestável, gerando uma nova fita que será usada como molde para dar início a um novo ciclo de amplificação. Ao final, após vários ciclos, é possível ter bilhões de cópias do fragmento original (KHAN, 2011). A qPCR é uma variação da técnica que permite amplificação e detecção simultâneas. Devido à sensibilidade, a qPCR é utilizada para diagnóstico de doenças, possibilitando a detecção de patógenos nos estágios iniciais de infecção, quando há uma carga pequena do patógeno, e consequentemente com baixa probabilidade de ser detectada por metodologias menos sensíveis. (WATHUO et al., 2017). No caso do RSV, um vírus de RNA, há uma etapa adicional com a enzima transcriptase reversa, capaz de sintetizar uma fita de DNA complementar (cDNA) ao RNA genômico viral, posteriormente amplificada (ALBERTS et al., 2017b). Embora a PCR

contorne o problema de sensibilidade, é uma técnica que necessita de equipamentos caros e muito específicos, além de levar um tempo maior para obtenção do resultado e precisar de mão de obra qualificada para operação (JAIN; SCHWEITZER; JUSTICE, 2022).

2.3 Biossensores

Os primeiros tipos de sensores utilizados foram os físicos, que precisaram ser aprimorados para coletar informações em novos sistemas de gerenciamento, ocasionando no surgimento de sensores químicos e biológicos para suprir essa demanda. Os sensores biológicos ou biossensores são dispositivos capazes de detectar um composto específico ou um grupo seletivo através de uma reação bioquímica entre o analito e um componente biológico acoplado a um transdutor, responsável por converter o sinal da reação em um sinal detectável, proporcional à concentração ou presença do analito (**Figura 4**) (SCHULTZ; TAYLOR, 1996).

Figura 4 – Constituição de um biossensor.



Fonte: Própria, 2022.

O primeiro biossensor foi desenvolvido em 1962 para a detecção de glicose, utilizando a enzima glicose oxidase acoplada em um transdutor eletroquímico (CLARK; LYONS, 1962), e desde então, são empregados em várias áreas distintas. Na área de alimentos, o sensor biológico é uma alternativa mais simples, barata e rápida para controle de qualidade de um produto do que as técnicas tradicionais, baseadas em análises químicas, espectroscópicas e cromatográficas (MEHROTRA, 2016). Um exemplo é a detecção de tiramina em alimentos fermentados, pois a alta concentração do composto significa a deterioração do alimento devido ao longo período de armazenamento (SOARES et al., 2019).

Biossensores aplicados na área ambiental possibilitam análise *in situ*, economizando tempo e gastos necessários em análises laboratoriais. Esses dispositivos são usados para

controle de qualidade de água, por exemplo, através da detecção de compostos danosos ao meio-ambiente e à saúde humana, como pesticidas (STÉFANNE E SILVA et al., 2020) e corantes da indústria têxtil (VENTURINI ULIANA; YAMANAKA, 2019).

Na área da saúde, os biossensores estão presentes, principalmente, no diagnóstico de doenças infecciosas, como a detecção do vírus Zika por meio da imobilização de RNA no transdutor (DA FONSECA ALVES et al., 2019). Outras aplicações incluem a identificação de pacientes no estágio final de insuficiência cardíaca e a descoberta de novos medicamentos (MEHROTRA, 2016).

2.3.1 Componentes biológicos

Várias moléculas biológicas podem ser imobilizadas na superfície do transdutor para o desenvolvimento do biossensor. As mais comuns são as enzimas, os ácidos nucleicos e os anticorpos. Outros componentes, como células inteiras e partículas virais também são utilizados, mas em menor proporção.

2.3.1.1 Enzimas

As enzimas, catalisadoras capazes de acelerar uma reação em milhões de vezes ou mais, são utilizadas para o desenvolvimento de biossensores, pois apresentam alta especificidade. A reação acontece quando o substrato se liga ao sítio ativo da enzima, formando o complexo enzima-substrato, e posteriormente há formação do(s) produto(s) (ALBERTS et al., 2017c).

O primeiro biossensor enzimático foi também o primeiro biossensor desenvolvido, ou seja, para a detecção de glicose através da imobilização da glicose oxidase (CLARK; LYONS, 1962). Desde então, as enzimas são usadas em sensores biológicos. As vantagens de utilizar uma enzima como componente biológico incluem a especificidade e a afinidade pelo substrato. Por outro lado, a maioria das enzimas são inibidas em certo ponto, quando há uma concentração muito elevada do substrato e/ou produto, além de efeitos no meio, como mudança na temperatura e no pH (SCHULTZ, 1996).

2.3.1.2 Ácidos nucleicos

Sensores biológicos de DNA, ou genossensores, utilizam o pareamento de bases entre duas fitas simples da molécula que forma uma fita dupla. Essa interação garante alta especificidade, que aumenta exponencialmente com o aumento de nucleotídeos do fragmento. Sendo assim, há a imobilização de DNA simples fita no transdutor e a detecção é feita a partir da hibridização com DNA complementar presente no analito (SCHULTZ, 1996; WANG, 2000). A detecção do vírus Influenza A é um exemplo de genossensor (DONG *et al.*, 2015).

Apesar do RNA ser encontrado em fita simples (exceção para alguns vírus) devido a sua instabilidade, o ácido nucleico também pode ser usado para desenvolvimento de biossensores, pois possui importante papel na medicina, já que muitos tipos de RNA são biomarcadores, pois sua desregulação é indicativa de doenças, como câncer. Portanto, são moléculas importantes para o desenvolvimento de novas formas de prognóstico e diagnóstico (ISLAM et al., 2017). Labib e colaboradores (2013) desenvolveram um biossensor para detecção de baixos níveis de microRNAs, baseado em hibridização, em ligação proteica e deslocamento de proteína para identificação de células cancerosas, por exemplo.

Os ácidos nucleicos ainda podem formar aptâmeros, fitas com sequências aleatórias com alta afinidade e especificidade de ligação com moléculas alvo, sendo uma alternativa a imobilização de anticorpos devido ao baixo custo, estabilidade, possibilidade de produção *in vitro* e imunogenicidade baixa ou inexistente, podendo ser usados na detecção de bactérias e vírus (LABIB et al., 2012).

2.3.1.3 Anticorpos

Os anticorpos são complexos proteicos sintetizados por linfócitos B quando o organismo entra em contato com um corpo estranho, denominado antígeno. Todos os anticorpos apresentam uma estrutura básica com pouca variabilidade, e uma região de ligação do antígeno com com enorme variabilidade, demonstrando a capacidade dessas moléculas de se ligarem em uma grande variedade de antígenos estruturalmente distintos. Os anticorpos são compostos por duas cadeias leves e duas cadeias pesadas idênticas, que apresentam regiões aminoterminais variáveis, responsáveis pelo reconhecimento do antígeno, e regiões carboxiterminais constantes, relacionadas às funções protetoras e efetoras desses complexos (ABBAS; LITCHTMAN; PILLAI, 2019a). Na maioria das vezes, os antígenos são moléculas muito maiores do que o sítio de ligação do anticorpo, de forma que possuam regiões específicas de

ligação, denominadas epítopos. A ligação antígeno-anticorpo é irreversível e altamente específica, reconhecendo antígenos que apresentam diferenças mínimas em sua estrutura e composição. Ademais, os anticorpos são amplamente diversos, de modo que um indivíduo consegue produzir grande quantidade desses complexos proteicos, com estruturas distintas (ABBAS; LITCHTMAN; PILLAI, 2019a).

Biossensores baseados em anticorpos, também denominados imunossensores, geralmente utilizam o isotipo IgG imobilizado no transdutor (HOCK, 1997; SCHULTZ, 1996). Devido à grande variabilidade da estrutura desse componente biológico, são aplicados em áreas distintas que vão da ambiental, como para detecção de corantes têxteis em rios (VENTURINI ULIANA; YAMANAKA, 2019), até a da saúde, como para diagnóstico de cânceres (CHAI et al., 2022).

2.3.1.4 Células inteiras

As células são as unidades fundamentais da vida. Todas elas são delimitadas por uma membrana, apresentam meio aquoso em seu interior (citoplasma) e têm capacidade de sintetizarem cópias de si mesmas (ALBERTS et al., 2017a; MADIGAN et al., 2016a). Os primeiros biossensores baseados na imobilização de células inteiras, sendo as procarióticas mais comuns, foram desenvolvidos para coletar informações sobre um estímulo em um organismo vivo, sendo aplicados na farmacologia, na biologia celular, na toxicologia e na área ambiental (BOUSSE, 1996). Atualmente, esses sensores também são usados em análises analíticas, como por exemplo, a imobilização da bactéria *Methylobacterium organophilium* para detecção de metanol (WEN et al., 2014) e a imobilização do fungo *Aspergillus nidulans* modificado geneticamente para detecção de estrogênio (ZUTZ et al., 2017).

2.3.1.5 Outras moléculas

Além das enzimas e anticorpos, outras proteínas que se ligam especificamente a uma molécula alvo são empregadas em sensores biológicos. As lectinas, por exemplo, presentes em sementes de raízes e leguminosas, interagem com carboidratos e são usadas na detecção de glicose, bactérias patogênicas, toxinas e células cancerosas (WANG; ANZAI, 2015). A detecção de *Escherichia coli* por um biossensor baseado na imobilização da lectina concanavalina A, é um exemplo descrito na literatura (GAMELLA et al., 2009).

A imobilização de bacteriófagos, isto é, vírus que infectam exclusivamente células bacterianas, também já foi feita. Richter e colaboradores (2017) demonstraram que bacteriófagos T4 podem ser utilizados para detecção de bactérias, em que *Escherichia coli* foi o microrganismo modelo do estudo.

2.3.2 Transdutores

Um dos componentes básicos do biossensor é o transdutor, responsável por converter o sinal oriundo da reação bioquímica envolvendo o analito para um sinal detectável. Existem vários tipos de transdutores utilizados para o desenvolvimento de sensores biológicos, de modo que o óptico e o eletroquímico são os mais comuns (SCHULTZ; TAYLOR, 1996).

2.3.2.1 Transdutores ópticos

Os transdutores ópticos monitoram as alterações como índice de refração, absorção, reflexão, comprimento de onda, fosforescência e fluorescência na luz no espectro do visível e do infravermelho advindas da reação bioquímica (DAMBORSKÝ; ŠVITEL; KATRLÍK, 2016; LEATHERBARROW; EDWARDS, 1999). Um exemplo é o método de ELISA, usado no diagnóstico de doenças, baseado na interação antígeno-anticorpo e na atividade catalítica de enzimas, como a peroxidase, a fosfatase alcalina e a β-galactosidase. A enzima, ligada ao anticorpo ou ao antígeno, reage com o substrato presente na amostra analisada e acarreta em um produto colorido quantificável, de maneira que a concentração do produto é sempre proporcional à intensidade da cor gerada (MADIGAN et al., 2016b).

2.3.2.2 Transdutores eletroquímicos

Biossensores eletroquímicos usam eletrodos que convertem o sinal da reação bioquímica em um sinal elétrico mensurável. Esse dispositivo une a vantagem da imobilização do componente biológico (especificidade) com as vantagens dessa transdução, que englobam sensibilidade e miniaturização. Eles podem ser classificados como amperométricos, potenciométricos, voltamétricos, condutométricos impedimétricos, de acordo com o tipo de sinal elétrico mensurado (WANG, 1996).

Os biossensores amperométricos se baseiam na mensuração de corrente elétrica originada pela oxidação e/ou redução eletroquímica de uma espécie eletroativa. A medida é

possível através do controle de potencial do eletrodo de trabalho (geralmente de platina, de ouro ou de carbono) em relação ao eletrodo referência (geralmente de prata/cloreto de prata). A corrente gerada mede diretamente a taxa da reação de transferência de elétrons, e consequentemente é proporcional à concentração do analito de interesse (THÉVENOT et al., 2001; WANG, 1996).

Transdutores potenciométricos convertem o sinal de reconhecimento em um potencial, que, de forma logarítmica, é proporcional a concentração da espécie gerada ou consumida durante a reação. Essa forma de medição proporcionou a expansão do uso de biossensores por não serem baseados no fluxo de elétrons, possibilitando maiores aplicações (WANG, 1996). Nesse caso, uma corrente nula é aplicada para mensurar o potencial entre um indicador e um eletrodo de referência ou dois eletrodos de referência, separados por uma membrana permeável seletiva. Dentre as medidas mais comuns com uso de dispositivos potenciométricos estão pH, além de outros íons (F^-, I^-, Cn^-, Na^+, K^+, Ca^{2+} e NH_4^+) e gases (CO_2 e NH_3) (THÉVENOT et al., 2001).

Biossensores voltamétricos utilizam de técnicas como voltametria cíclica, voltametria de pulso diferencial e voltametria de onda quadrada para a detecção do analito, que ocorre a partir da mudança na corrente e no pontencial aplicado, ou seja, há medição da corrente e do potencial. O valor da corrente do pico é usado para identificação do analito, de forma que a densidade da corrente do pico é proporcional à concentração do composto correspondente (SRIVASTAVA *et al.*, 2020).

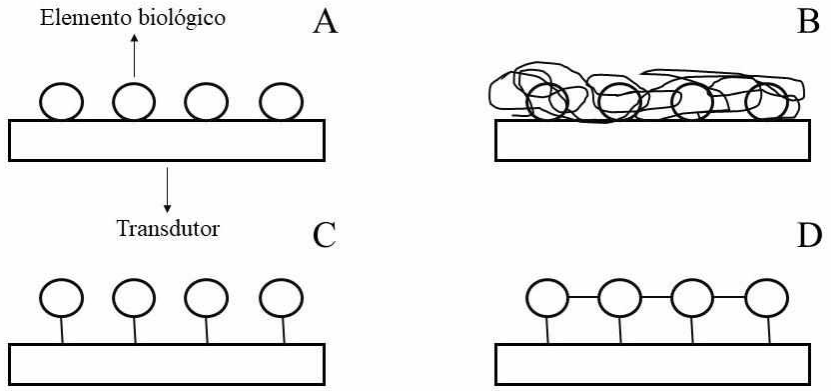
A espectroscopia de impedância eletroquímica (EIS) e a condutometria são duas técnicas relacionadas à medição de propriedades elétricas de soluções, porém possuem diferenças distintas em suas abordagens e aplicações. A condutometria é focada na medição direta da condutividade elétrica de uma solução, relacionada à capacidade de condução de corrente pelos íons presentes nessa solução. É uma técnica simples e direta, amplamente utilizada para determinar concentrações de íons em soluções aquosas. Por outro lado, a EIS é uma técnica mais avançada e abrangente que analisa a resposta elétrica de interfaces eletroquímicas. Ela envolve a aplicação de uma excitação senoidal de frequência variável e a medição da resposta em termos de impedância elétrica em diferentes frequências. A EIS fornece informações detalhadas sobre as propriedades de interfaces eletroquímicas, como resistência, capacitância e reatâncias, permitindo o estudo da cinética de reações, transferência de carga e comportamento de adsorção em sistemas eletroquímicos complexos. Em resumo, enquanto a condutometria é mais simples e voltada para a determinação de concentração de íons, a EIS é

uma técnica avançada que permite uma análise mais detalhada das interfaces eletroquímicas. (BOTT, 2001; GUAN; MIAO; ZHANG, 2004).

2.3.3 Técnicas de imobilização

A etapa mais importante no desenvolvimento de um biossensor é a imobilização eficiente do componente biológico na superfície do transdutor. Existem várias técnicas para realizar esse procedimento, cada uma possuindo suas vantagens e desvantagens específicas. A **Figura 5** mostra esquematicamente os principais tipos de imobilização.

Figura 5 – Técnicas de imobilização do componente biológico no transdutor; (A) adsorção física, (B) oclusão, (C) ligação covalente e (D) *cross-linking*.



Fonte: Própria, 2022.

2.3.3.1 Adsorção física

A adsorção física, mostrada na **Figura 5A**, é a técnica mais antiga e mais simples de imobilização conhecida. Ela é baseada em interações não covalentes entre a superfície do transdutor e o componente biológico, como *Van der Walls*, eletrostática, hidrofóbica e hidrofílica (BHARDWAJ, 2014). Nessa metodologia, geralmente é preparado uma solução do componente biológico que será imobilizado em uma membrana ou filme para possibilitar a adsorção no transdutor. A membrana ou filme pode apresentar grupos iônicos e/ou ter característica hidrofílica ou hidrofóbica, de acordo com as propriedades do componente biológico (TAYLOR, 1996). O primeiro biossensor desenvolvido foi baseado na imobilização da glicose oxidase em uma membrana de polietileno (CLARK; LYONS, 1962). Apesar de sua simplicidade no processo de imobilização, a adsorção física é bastante sensível a mudanças de

pH, de temperatura e de quantidade de íons na amostra, fazendo com que o componente biológico possa ser lixiviado mais facilmente da superfície do transdutor em comparação com outras técnicas (TAYLOR, 1996).

2.3.3.2 Oclusão

A oclusão (**Figura 5B**) foi desenvolvida para garantir a imobilização efetiva de moléculas lábeis. Nessa técnica, o componente biológico é preparado juntamente com os constituintes de uma membrana ou filme, ou é difundido após a formação da membrana ou filme na superfície do transdutor, de modo que fique aprisionado, sem ligação química (TAYLOR, 1996). Um exemplo é um dos primeiros biossensores enzimáticos desenvolvidos, o qual houve o oclusão da enzima urease em acrilamida para a detecção de ureia (GUILBAULT; MOLTAVO JR, 1969). Mesmo que esse método consiga aprisionar componentes lábeis através da membrana ou filme, ele consiste em uma interação fraca que pode ser interrompida em condições diversas. Outra desvantagem da oclusão está no fato de que a membrana ou filme pode impedir que o componente biológico interaja com o analito de interesse, pois podem ocupar a região de ligação da molécula (TAYLOR, 1996).

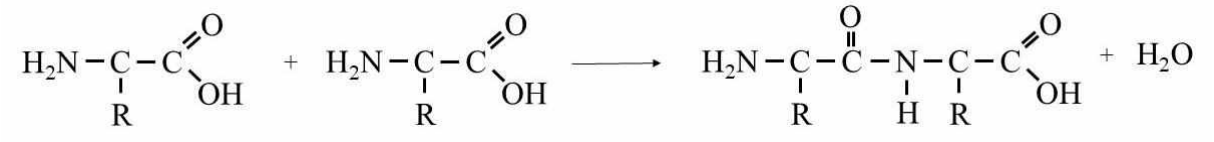
A oclusão pode ser feita por sol-gel, um procedimento de síntese de materiais que transita de um sistema sol, isto é, partículas coloidais com 1-100 nm dispersas de forma estável em um fluido, para um sistema gel, ou seja, uma estrutura rígida formada pelas partículas coloidais ou cadeias poliméricas, de maneira que a parte líquida fique contida nos poros do material (ALFAYA; KUBOTA, 2002). A detecção de galactose pela oclusão de galactose oxidase em um filme de Nafion é um exemplo de biossensor que utiliza o sistema sol-gel (LEE et al., 2021). Outra forma de ocluir o componente biológico é através da formação de um polímero na superfície do transdutor utilizando técnicas eletroquímicas, como por exemplo, a imobilização de glicose oxidase através de um polímero derivado de *m*-fenilenodiamina (ALMEIDA; WINGARD; MALMROS, 1990).

2.3.3.3 Ligação covalente

Na ligação covalente (**Figura 5C**), o transdutor tem sua superfície modificada com uma membrana ou filme que possua grupos funcionais disponíveis para que ocorra a ligação química com o componente biológico. A ligação peptídica, responsável pela ligação de aminoácidos para formação de uma proteína (**Figura 6**), é uma forma bastante utilizada de ligação covalente.

Nesse tipo de imobilização os grupos nucleofílicos da molécula a ser imobilizada (geralmente uma proteína) atacam grupos ativados presentes no transdutor. A principal vantagem da técnica é a maior resistência a mudanças de pH, temperatura e força iônica. Tal imobilização pode acarretar em perdas da atividade do componente biológico, principalmente para moléculas lábeis, além de seu preparo ser mais demorado quando comparado com a adsorção física e a oclusão (TAYLOR, 1996).

Figura 6 – Formação de uma ligação peptídica entre dois aminoácidos. A ligação ocorre entre um grupo amino primário (-NH_2) e um grupo carboxila (-COOH).



Fonte: Própria, 2022.

Além da modificação do transdutor, é necessário ativar os grupos funcionais que estarão disponíveis para ligação com o componente biológico. Para ligações peptídicas, com necessidade de ativar o grupo carboxila presente no transdutor, é comum o uso de uma solução de 1-etil-3-(3-dimetilaminopropil)carbodiimida (EDC) e N-hidroxisuccinimida (NHS) para formação de ésteres estáveis capazes de conjugar com os grupos aminos primários do componente biológico (STÉFANNE E SILVA et al., 2020; VENTURINI ULIANA; YAMANAKA, 2019).

2.3.3.4 Cross-linking

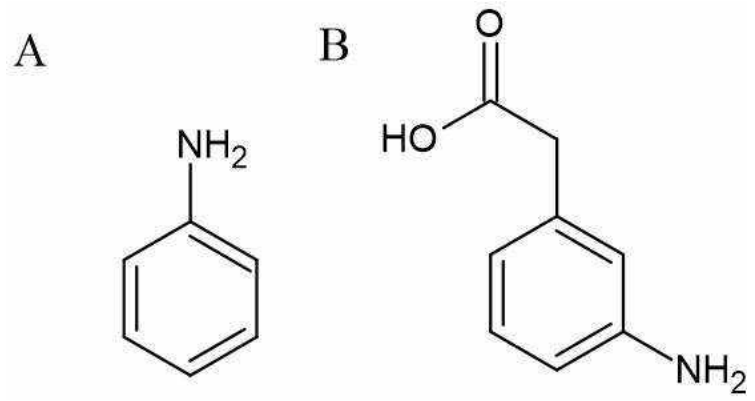
O *cross-linking* (**Figura 5D**) une as características da oclusão e da ligação covalente, em que há formação de um filme na superfície do transdutor que consiga fazer, simultaneamente, o aprisionamento e a ligação química com o componente biológico. Dessa forma, essa técnica é mais resistente a mudanças de pH, temperatura e força iônica em comparação aos métodos baseados em interações físicas e consegue imobilizar moléculas lábeis de forma efetiva. Em contrapartida, assim como na oclusão, o sítio de ligação do componente biológico pode se tornar inativo, além de ser mais trabalhosa (TAYLOR, 1996). Um dos compostos mais comuns no *cross-linking* é o glutaraldeído, que já demonstrou ser capaz de imobilizar anticorpos (TAYLOR; MARENCHIC; SPENCER, 1991) e enzimas (BARBOSA et al., 2014) com sucesso.

2.4 Polímeros condutores

Polímeros são comumente usados para modificar a superfície do transdutor eletroquímico para que a imobilização do componente biológico seja efetiva. Polímeros condutores são compatíveis com moléculas biológicas em solução aquosa de pH neutro e conseguem transferir as cargas elétricas oriundas de reações bioquímicas para um circuito elétrico de forma eficiente (TAHIR; ALOCILJA; GROOMS, 2005). Polímeros condutores combinam propriedades elétricas dos metais e as propriedades mecânicas dos polímeros. Esses materiais apresentam uma cadeia principal com ligações simples (σ) e ligações duplas (π) alternadas. As ligações simples são fortes e possuem um maior número de elétrons localizados, enquanto as ligações duplas são fracas e possuem menos elétrons localizados, e consequentemente, permitem uma maior mobilidade. Entretanto, para que um polímero seja efetivamente condutor, é necessário que os elétrons sejam removidos por dopagem, formando vacâncias, que são preenchidas por outros elétrons da estrutura, gerando novas vacâncias (KUMAR; SHARMA, 1998; MEDEIROS et al., 2012).

Polímeros condutores provenientes de monômeros que apresentam anel aromático, como a anilina, melhoram a performance do biossensor e impedem a adsorção de interferentes na superfície do transdutor (**Figura 7A**), que gerou o primeiro polímero condutor a ser comercializado e já possui sua eletropolimerização muito bem estudada devido ao grande número de aplicações, incluindo o desenvolvimento de biossensores eletroquímicos (DHAND et al., 2015).

Figura 7 – Estrutura química da (A) anilina e do (B) 3APA.



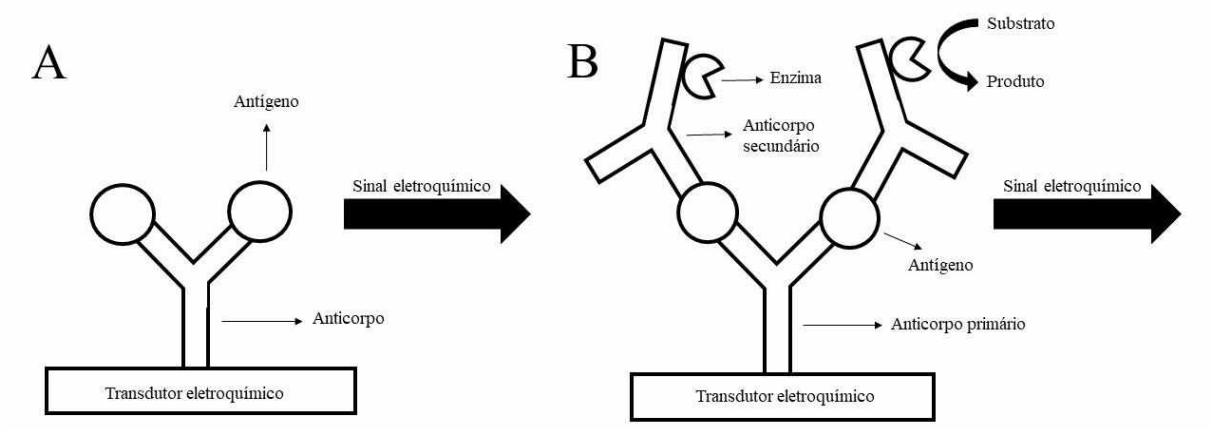
Fonte: Própria, 2022.

Monômeros derivados da anilina são possíveis candidatos a formarem polímeros condutores, como o ácido 3-aminofenilacético (3APA) (**Figura 7B**), com fórmula molecular $C_8H_9NO_2$ e peso molecular 151,16 $g.mol^{-1}$, que apresenta um grupo carboxila, um grupo amino e um anel aromático (NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION, 2022), características encontradas em monômeros com aplicabilidade no desenvolvimento de biossensores. O grupo carboxila, não ligado diretamente ao anel aromático, normalmente não é suscetível à eletropolimerização, ou seja, fica disponível para a ligação covalente com o elemento biológico. (ALVES-BALVEDI et al., 2016). O monômero apresenta um grupo amino ligado diretamente ao anel aromático, que está envolvido no mecanismo de ação de sua polimerização eletroquímica.

2.5 Imunossensores eletroquímicos

Os imunossensores eletroquímicos exploram a ligação antígeno-anticorpo para detecção, de modo que um deles seja imobilizado na superfície do transdutor. A detecção do analito pode ser feita de forma direta (**Figura 8A**), através da medida eletroquímica gerada pela ligação antígeno-anticorpo, ou, após a interação entre o componente biológico imobilizado e o analito, havendo um segundo anticorpo, marcado com enzima, incorporado ao sistema, juntamente com um substrato enzimático adequado (**Figura 8B**). Dessa forma, a reação enzimática gera um produto que fornece uma medida eletroquímica (RICCI; ADORNETTO; PALLESCHI, 2012).

Figura 8 – Componentes básicos de um imunossensor eletroquímico com detecção (A) direta e (B) com anticorpo secundário marcado.



Fonte: Própria, 2022.

A performance de polímeros condutores em imunossensores pode ser vista no trabalho de Lorenzen e colaboradores (2022), através da imobilização da nucleoproteína do vírus SARS-CoV-2 para diagnóstico de COVID-19, pela detecção do anticorpo na amostra de pacientes. Desse modo, o eletrodo de trabalho composto por aço foi modificado com polímero derivado de 3,4-etilenodioxitiofeno e nanopartículas de ouro. Outro trabalho, desenvolvido por Palomar (2018) e colaboradores também utilizou um polímero condutor para melhorar resposta em um biossensor impedimétrico para detecção de anticorpos de dengue. Para isso, foi usado eletrodo de carbono vítreo modificado com nanotubos de carbono e polímero condutor derivado de pirrol, para a imobilização da proteína NS1 do vírus da dengue.

CAPÍCULO 2: ARTIGO CIENTÍFICO

Development of a label-free impedimetric immunosensor for the detection of respiratory syncytial virus

Tallita Stéfanne e Silva[a], Guilherme Ramos Oliveira e Freitas[b], Lucas Franco Ferreira[c], Diego Leoni Franco[a*]

[a] Group of Electrochemistry Applied to Polymers and Sensors - Multidisciplinary Group of Research, Science, and Technology (RMPCT), Laboratory of Electroanalytical Applied to Biotechnology and Food Engineering (LEABE) – Chemistry Institute, Federal University of Uberlândia – campus Patos de Minas, Rua Major Jerônimo, 566, 38.700-002, Patos de Minas, Minas Gerais, Brazil

[b] Laboratory of Microbiology (MICRO), Biotechnology Institute, Federal University of Uberlândia –campus Patos de Minas –Av. Getúlio Vargas, 230, 38.700-128, Patos de Minas, Minas Gerais, Brazil

[c] Institute of Science and Technology, Laboratory of Electrochemistry and Applied Nanotechnology, Federal University of the Jequitinhonha and Mucuri Valleys, Diamantina, Minas Gerais, Brazil

tallita.stefanne@ufu.br, grofreitas@ufu.br, lucas.franco@ict.ufvjm.edu.br

*Corresponding author. *E-mail address:* diegoleoni@ufu.br (D.L. Franco)

Abstract: Respiratory syncytial virus (RSV) is an important pathogen responsible for cases of bronchiolitis, bronchitis, and pneumonia, worryingly effective and lethal to children under the age of five. Current detection methods for RSV are expensive, demand time, and high-skilled personnel. Therefore, in this work, we developed a cost-effective impedimetric immunosensor for the detection of RSV based on a pencil graphite electrode (PGE) modified with a polymeric film derived from 3-amino phenylacetic acid (3APA). The polymer was formed and characterized through cyclic voltammetry. The current response decrease in potassium ferricyanide solution and the current response increase in methylene blue solution suggest the presence of unchanged carboxylic groups. The number of electrons involved in the first step of the reaction and the electron/proton ratio were like the one proposed for aniline, and a mechanism of electropolymerization was proposed. The immunosensor was assembled by the activation of the carboxyl groups, plunging the modified electrode in an EDC/NHS solution, followed by a nucleophilic substitution reaction with the amino groups present in the anti-RSV antibody (Ab). The surface was blocked with glycine before the incubation with the virus. Detection was performed using electrochemical impedance spectroscopy (EIS), and the data were evaluated using equivalent circuits. The values of resistance of charge transfer (Rct) increased as the PGE was modified in the following order: PGE < PGE/polymer < PGE/polymer/Ab < PGE/polymer/Ab/virus. For optimization, 100 ng of Ab was used, and the immobilization time for Ab and Gly were: 3 h and 50 min, respectively. The response time of the system, with Ag immobilization, was 30 min. The limit of detection was 27.65 $PFU.mL^{-1}$, and the limit of quantification was 92.15 $PFU.mL^{-1}$, with a linear range from 50 – 1000 $PFU.mL^{-1}$, demonstrating that the proposed immunosensor is a viable alternative for the detection of RSV.

Keywords: Respiratory syncytial virus, biosensor, electropolymerization

1. Introduction

Acute respiratory infections are characterized by the obstruction of air passage in the respiratory system, and it is one of the major causes of illness and death, especially in children. The respiratory syncytial virus (RSV) is one of the most important pathogens responsible for this scenario, especially in children up to five years old. It is characterized by failure to develop long-term immunity, leading to lifelong reinfections. In 90% of cases, the first contagion occurs until the first two years of life [1], and in severe cases, it can lead to pneumonia and bronchiolitis [2].

It is a contagious virus, as it enters the host organism by the conjunctiva-upper respiratory tract mucous through direct contact or contaminated droplets. After the incubation time (2-8 days), it spreads until it reaches the epithelium, where its growth is favored [1,3]. Roughly 30 million new cases of RSV infection occur annually, with more than 100 thousand deaths of children. Developing countries present a higher mortality rate, probably because of the late diagnosis, corresponding to 90 % of all deaths caused by the virus [4].

Generally, diagnosis is performed through physical exams based on the patient's symptoms. However, because of the high number of different microorganisms that can generate the same symptoms, the precise identification of RSV is only possible through laboratory analysis. The indirect immunofluorescence assay is the most common method with high sensitivity [5], the immunochromatographic assays, also known as rapid tests, provide results within a 10 to 30-minute range and are cost-effective [6], and the RT-qPCR is the gold standard method, in substitution of the virus isolation in cell culture [7]. However, there is a demand for a method that can rapidly determine the virus specifically with high sensitivity and cost-effectiveness in a friendly-used device, all advantages usually found in biosensors.

The structure and physiopathology of RSV are well-settled in the literature [8], as well its immunological response [9], with a library of specific antibodies available as the pivotal material for the effective response of the immunoassays and strong candidates for an immunosensor development. Electrochemical impedance spectroscopy (EIS) is a feasible choice and a powerful tool [10] for label-free RSV determination based on the strong antibody-antigen interaction through interfacial properties changes, and it has been useful for biosensor development [11–13]. The efficient biomolecule immobilization over the transducer surface is a key-step in the device assembly, and functionalized polymers

electrogenerated over electrodes are fulfilling this requirement by covalently attaching the biomolecules through the available functional groups [14–16]. In this sense, the monomer 3-amino phenylacetic acid (3APA) is an excellent candidate as a polymeric matrix. The carboxylic acid, away from the ring by a methyl group, tends to remain unchanged and free to bind to the biomolecules through a covalent bond, while it is expected that the polymerization begins from the nitrogen atom like the well-known mechanism for polyaniline [17].

Therefore, this work focused on the electropolymerization of 3APA over pencil graphite electrodes (PGE) and the characterization of the formed product, followed by the development of a label-free impedimetric immunosensor for RSV determination. To the best of our knowledge, this is the first attempt to develop an impedimetric immunosensor for RSV using this polymeric matrix over PGE.

2. Materials and methods

2.1. Chemicals

All solutions were prepared using deionized water, and all the chemicals were used as received. Sulfuric acid (H_2SO_4) was obtained from LS Chemicals®, potassium chloride (KCl) and potassium dihydrogen phosphate (KH_2PO_4) from Dinâmica®, potassium ferricyanide ($K_3Fe(CN)_6$), boric acid (H_3BO_3), and sodium hydrogen phosphate (Na_2HPO_4) from Vetec®, methylene blue ($C_{16}H_{18}ClN_3S$) and dimethylsulfoxide (DMSO) from Isofar®, 3APA, 2-methanessulfonic acid (MES) ($C_6H_{13}NO_4S$), EDC ($C_8H_{17}N_3$), and NHS ($C_4H_5NO_3$) from Sigma-Aldrich®, acetic acid (CH_3COOH) and phosphoric acid (H_3PO_4) from Labsynth®, sodium hydroxide (NaOH) from Synth®, Anti-RSV Antibody, fusion protein, all type A, B strains, clone 131-2A from Merck Millipore® (MAB8599), sodium chloride (NaCl), Glycine ($C_2H_5NO_2$), and sodium bicarbonate ($NaHCO_3$) from Êxodo Científica®, fetal bovine serum (FBS), and *Dulbecco's* Modified Eagles (DMEM) medium from Gibco®, penicillin 10000 $\mu g.mL^{-1}$ and streptomycin 10000 $U.mL^{-1}$ from Hyclone®.

2.2. Equipment

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) experiments were performed in a potentiostat model PGSTAT204 with an FRA module from Metrohm (Netherlands). PGE mines from Pentel, hardness HB, named Super Hi-Polymer C505, with a diameter of 0.9 mm, were used as working electrodes. To ensure reproducibility, the area of the electrode was kept constant at 0.289 cm^2 for all experiments. Platinum wire and Ag/AgCl (KCl 3.0 M) electrodes were used as auxiliary and reference electrodes. The PGEs were mechanically polished using sandpaper, followed by washing in deionized water, ultrasonication, and drying using ultra-pure N_2 gas.

2.3. Electropolymerization and characterization

The electropolymerization of 3APA (1.0 mM) was performed using sulfuric acid (0.10 M) as a supporting electrolyte through CV (0.05 V s^{-1}). The voltammograms were recorded from 0.0 to 1.2 V. The system was analyzed through CV in KCl (0.5 M), $K_3Fe(CN)_6$ (5,00 mM) in KCl (0,50 M), $C_{16}H_{18}ClN_3S$ (5,00 mM) in KCl (0,50 M), and in H_2SO_4 (0,10 M) solutions to evaluate the modification of the electrode and to characterize the material. EIS measurements were performed in a $K_3Fe(CN)_6$ (5.00 mM) in KCl (0.50 M) solution, frequency range from $1x10^5$ to $1x10^{-2}$ Hz, $1x10^{-2}$ V_{RMS}; frequency number = 60; open circuit potential.

2.4. Virus and cell culture

Respiratory syncytial virus, subgroup A, strain A2, were obtained from Laboratório de Cultura de Células Animais, Universidade Federal de Uberlândia, campus Patos de Minas. The virus was propagated in HEp-2 cells (ATCC CCL-23) in Dulbecco's modified Eagle medium (DMEM) free of antibiotics and fetal calf serum (FCS), and the supernatant was used in the titration and Immunoelectrochemical assay. Culture supernatant from un-infected HEp-2 cells was processed similarly to use for mock virus preparation. $0,5x10^7$ PFU mL^{-1} of RSV titration was determined in plaque forming units (PFU) assay. Subsequently, the virus was inactivated by heating at 100 °C for 15 min and was stored at −80 °C for further use.

2.5. Immunosensor assembly and RSV determination

Anti-RSV and RSV stock solutions (50 ng mL^{-1} and $5.00x10^6$ PFU mL^{-1}, respectively) were diluted in PBS 1x, pH 7.4 before each experiment. The carboxyl groups of the polymer were first activated, plunging the modified electrode in an EDC (0.1 M) and NHS (0.02 M) in MES (0.1 M, pH 4.5) aqueous buffer solution for 30 minutes. After, the electrode was plunged in a 15.0 µL diluted antibody solution (6.67 ng μL^{-1}) for 3 hours. The sensor surface was blocked with glycine (50.0 mM) in PBS 1x, pH 7.4, for 50 minutes, as previously reported [18]. Lastly, the immunosensor was plunged into a 15.0 µL diluted RSV solution for 30 minutes. Washing was performed after each step. The RSV was determined through EIS in the same configuration presented in section 2.3.

2.6. Optimization and validation

The impedimetric immunosensor was optimized (n=3) regarding the amount of antibody, time of antibody, glycine, and RSV incubation. The limit of detection (LOD) was calculated using equation $3s_b/a$, and the limit of quantification (LOQ) was calculated using the equation $10s_b/a$, where s_b is the standard deviation of a blank solution, and a is the slope of the linear equation obtained from the calibration curve at different RSV concentrations. As the virus was obtained from cell culture, cell culture in the absence of the virus was tested to discard interference of the matrix on the positive response.

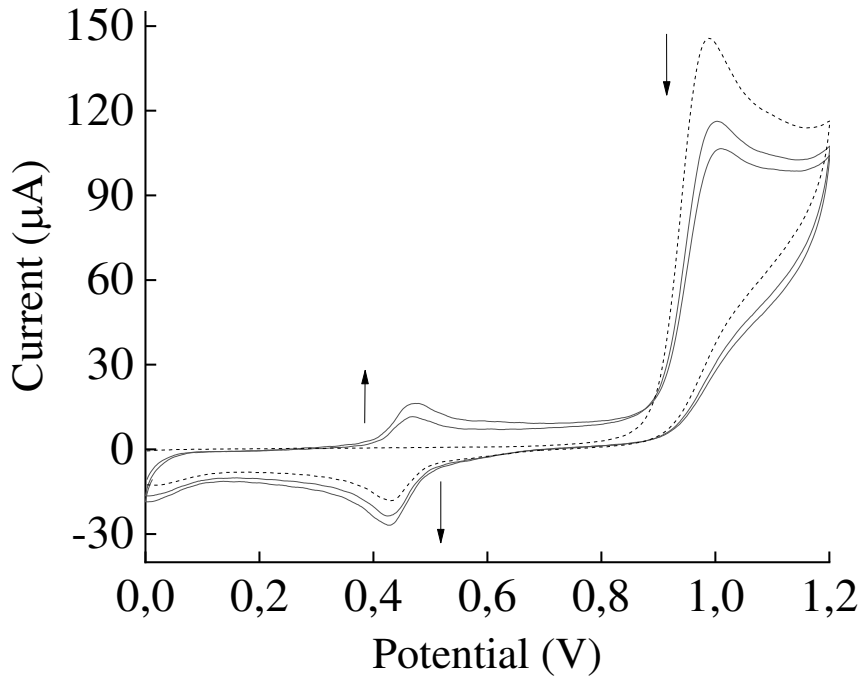
3. Results and discussion

3.1 Electropolymerization and characterization

The first three scans obtained in a 3APA/sulfuric acid solution over PGE performed through CV resulted in a faradaic anodic response at +0.99 V, followed by a redox couple signal at +0.43/+0.48 V (**Fig.1**). The first signal results from an irreversible monomer oxidation, expected to occur on the nitrogen atom to generate the first radical cation. The current response decreases with subsequent scans with a slight shift to a more anodic potential due to the surface modification with a material hampering the diffusion

of new monomer species toward the electrode. The redox couple presents a current increase with subsequent scans, suggesting a material growth over the electrode surface. These responses at a more cathodic potential show the easiness in the electron transference to and from the electrode, as expected from novel aromatic structures with more mesomeric forms. This analysis is under the classic responses obtained from aniline [19] and derivatives [20,21] electropolymerization in acid conditions.

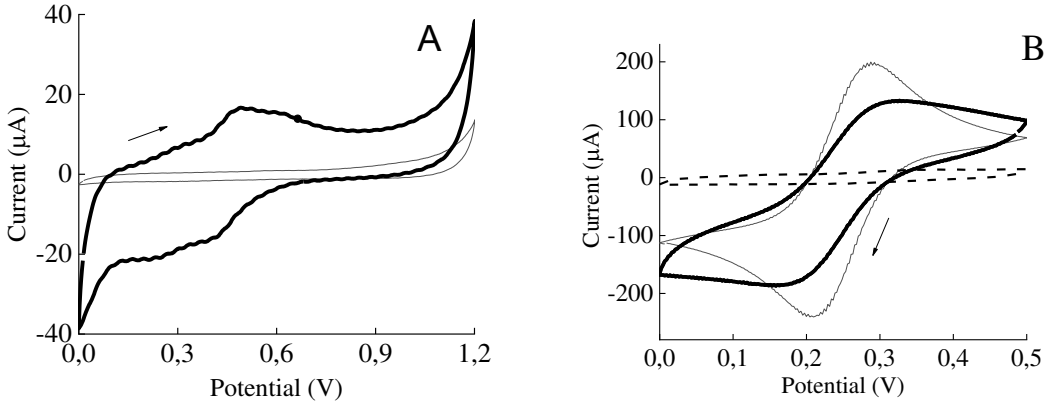
Fig.1. CVs recorded in 3APA (2.5 mM) using H_2SO_4 (0.5 M) as supporting electrolyte over PGE, 50 mV s^{-1}, 3 cycles. The arrows indicate the increase/decrease of the current response. The first scan is represented by a dashed line.



The electrode modification was first evaluated in the blank supporting electrolyte solution (**Fig.2A**), where it is possible to confirm the absence of the monomer oxidation signal at more anodic potential and the persistence of the redox couple current signal. The presence of the carboxyl groups in the material can be assessed by the voltammograms performed in a ferricyanide solution and a methylene blue (MB) solution. The KCl aqueous solutions present a pH value of around 6-7, in which the carboxylate anions are predominant. Therefore, it is expected an electrostatic repulsion between carboxylate and ferricyanide, as well as an electrostatic attraction between the charged polymer and MB. The current decrease observed in **Fig.2B** and the current increase observed in **Fig.2C** strengthen the repulsion/attractive hypothesis, suggesting the presence of the functional

group alongside the polymeric chain, which is essential for biosensor development. The Nyquist diagram presented in **Fig.2D**, and the experimental EIS data fitted through the Randles circuit (**Table 1**), corroborate the data obtained in the ferricyanide solution with a 5.32 times increase in R_{ct} values from PGE (89.68 Ω cm^{-2}) to the modified PGE (477.20 Ω cm^{-2}). The R_s are practically the same for both systems, showing good reproducibility. There is also a 19.86 times increase in Q_{dl}, suggesting the presence of a more compact material over PGE, filling the graphite gaps and not only the outer shell. Moreover, this difference might be related to the amount of charge present in the electrode surface, which could be resulted from the charged carboxylate anions. The Warburg resistance is similar between the systems, which indicates that the mass transport is unaltered with the electrode modification. The electrode modification was optimized with the number of cycles. It was observed that 30 cycles are an optimum number of cycles based on the small differences in the electrochemical profile performed in potassium ferricyanide and sulfuric acid solutions from this point on. Also, the system was kept stored at -4°C. After ten months, the modified electrode retained 91 % (anodic current) and 83 % (cathodic current) of its initial ferricyanide response, which proves that the material is stable with storage time and can be used for further biosensor development.

Fig.2. CVs recorded in (A) H_2SO_4 (0.5 M) at 50 mV s^{-1}, (B) $K_3Fe(CN)_6$ (5.00 mM) in KCl (0.50 M) at 100 mV s^{-1}, (C) methylene blue (5.00 mM) in KCl (0.5 M) at 100 mV s^{-1}, and (D) Nyquist diagram recorded in $K_3Fe(CN)_6$ (5.0 mM) containing KCl (0.1 M). OCP at 0.25 V; ΔE = 10 mV; Frequency range: 100,000 Hz to 10 mHz over PGE (line/open circle for D), and poly-(3APA)-modified PGE (bold lines, gray circles in D). Dashed lines in B and C represent measurements recorded in KCl (0.5 M) for PGE. Solid lines in D represent the fit of the equivalent circuit to the experimental findings. Inst in figure D shows the Randles circuit used to fit the data. The arrows indicate the initial scan direction.



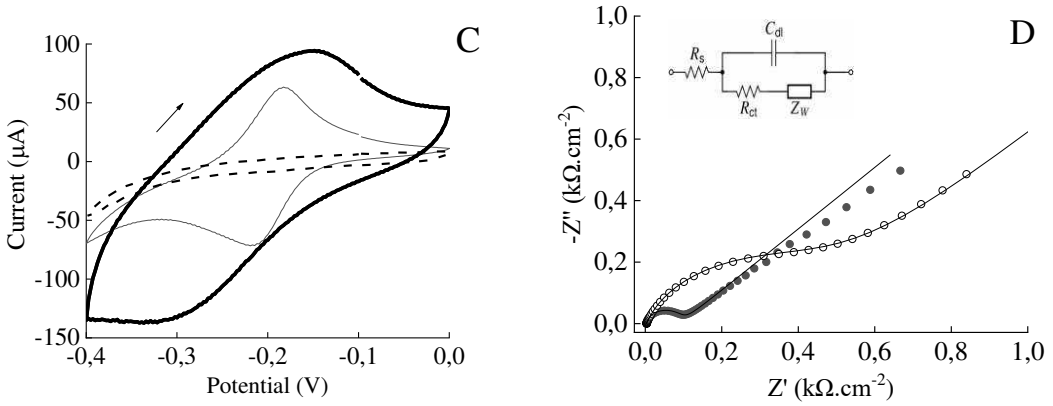


Table 1. Parameters obtained from EIS data simulation for PGE and modified PGE using Randles circuit.

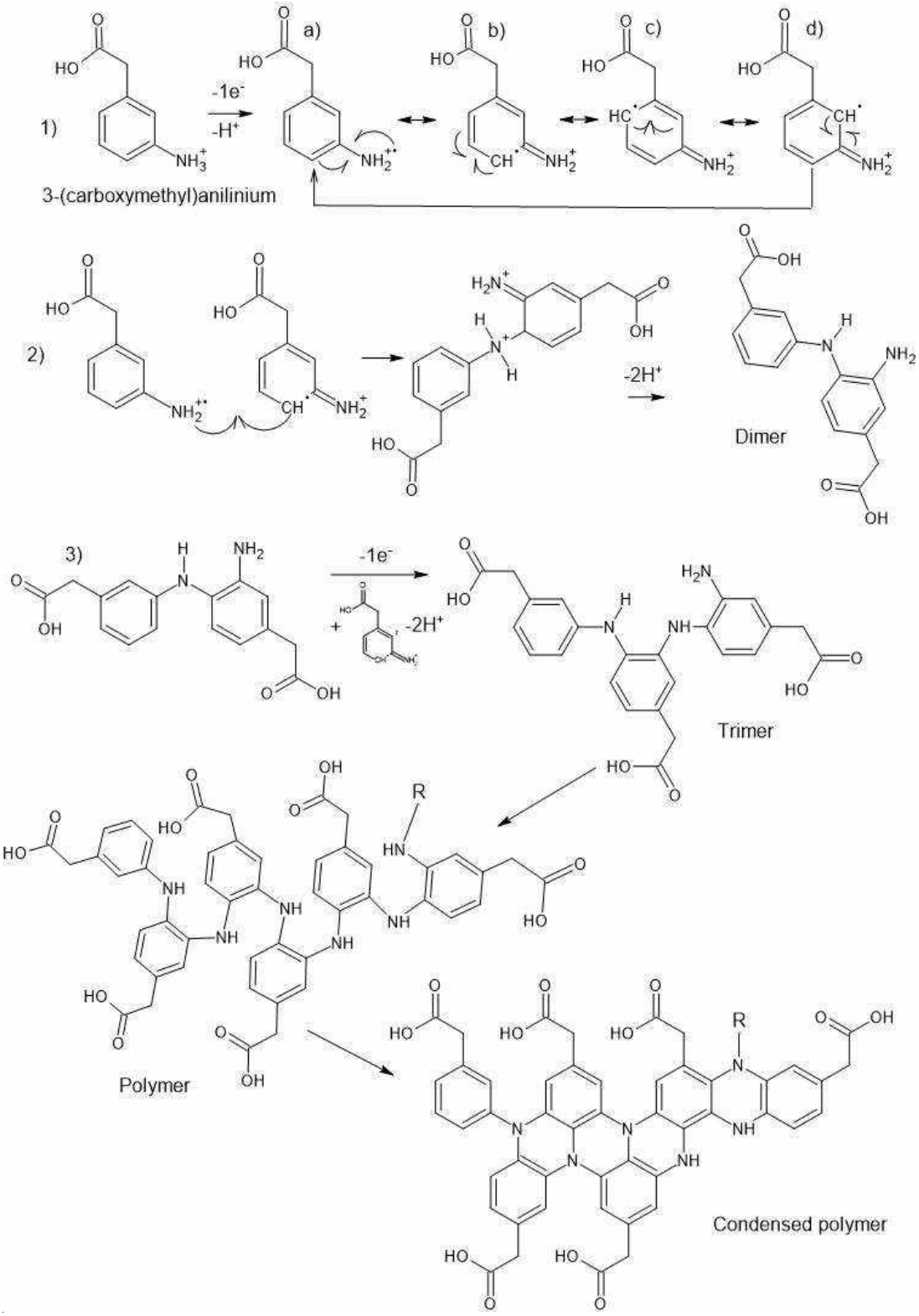
Parameter	PGE	PGE/poly-(3APA)
Rs (Ω)	11.36	12.01
Rtc ($\Omega.cm^{-2}$)	89.68	477.20
Qdl ($m\Omega.s^{N}$)	$6.67x10^{-6}$	$1.32x10^{-4}$
N	0.93287	0.79756
Zw ($m\ \Omega\ .s^{1/2}$)	$1.42x10^{-3}$	$1.16x10^{-3}$
X^2	0.0115	0.0423

3.2 Mechanism proposal

Electrochemical equations can provide useful information to understand the mechanism of 3APA electropolymerization, as was previously reported [14]. Cyclic voltammograms were recorded in the acidic monomeric solution changing the scan rate. Linear regression from plots of the log of peak current *vs* peak potential (**Fig.S1A)** and peak potential *vs* log of scan rate (**Fig.S1B)** were applied. The Tafel and Laviron equations were used based on the slope obtained from each study, thus providing the electron transfer coefficient (α) and the number of electrons (n), respectively. For an α of 0.445 for 3APA, the number of electrons calculated was 1.55. Because of the similarity of the monomer with aniline, it is more likely that the first step of the reaction involves one electron than two. Monomer solutions were also prepared in BR buffer at different pH values, and a voltammogram of the first scan was recorded in each solution. The peak potential *vs* pH profile was obtained (**Fig.S2**) with a linear behavior between pH 2 and 5 followed by a plateau, indicating no structural changes after this point, which matches the second monomer pKa. According to the Nernst equation [22], with a linear slope of 0.05598, it is believed that the monomer's first oxidation occurs with the involvement of

the same number of protons and electron, more precisely, one proton and one electron, based on the previous study. Thus, with the information gathered through the electrochemical experiments, an electropolymerization mechanism was proposed (**Fig 3**).

Fig.3. Electropolymerization mechanism proposal for poly-(3APA).



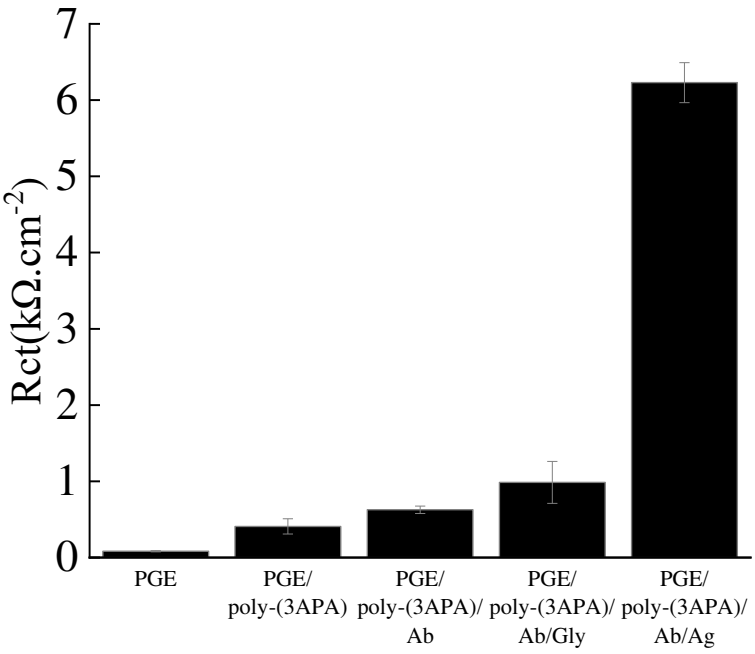
In strong acidic media, 3APA is protonated in the form of 3(carboxymethyl)anilinium cation. Step 1 is the electrochemical withdrawal of one electron and one proton from the ammonium group to generate the radical cation stabilized in 4 mesomeric forms (a to d). Among them, structure (b) presents less steric hindrance compared to structure (c) because of the proximity of the radical with the carboxyl group and to structure (d) with the radical between two bulky groups. Step 2 is the homolytic reaction between structures (a) and (b), followed by the withdrawal of two protons by the solvent, regenerating an aromatic in the form of a neutral dimer. This structure is believed to be responsible for the redox couple response during electropolymerization at less anodic potentials because of the easiness of electron removal from a structure with more mesomeric forms. After subsequent reactions (step 3), a polymer is formed. Here, we present two possible polymer structures. Based on the proximity of the nitrogen atom to the aromatic ring, it can be excepted a formation of condensate rings, improving the material stability. Regardless of the final structure, both present the same desired array, with the unchanged carboxyl group, which will be used for further reaction with the antibody.

3.3 Biosensor development

EIS was performed after each step of the biosensor construction, and the data was fitted using the Randles circuit. The R_{ct} was used as the parameter of analysis for all the experiments (**Fig 4**). It is expected an increase in the values of resistance of charge transfer after each electrode modification. The resistance increase from the polymer (477.20 Ω cm^{-2}) to the antibody (642.16 Ω cm^{-2}) suggests that the biomolecule was efficiently immobilized through the covalent bond. Even though antibodies are large molecules, the resistance increase is not substantial because we only used 100 ng of the biomaterial. The resistance increases with further surface blocking with glycine (957.20 Ω cm^{-2}), avoiding non-specific adsorption over the electrode surface. Then, a 1.8 times resistance increase (1727.27 Ω cm^{-2}) was observed with the virus, proving the biosensor efficiency in the presence of the antigen.

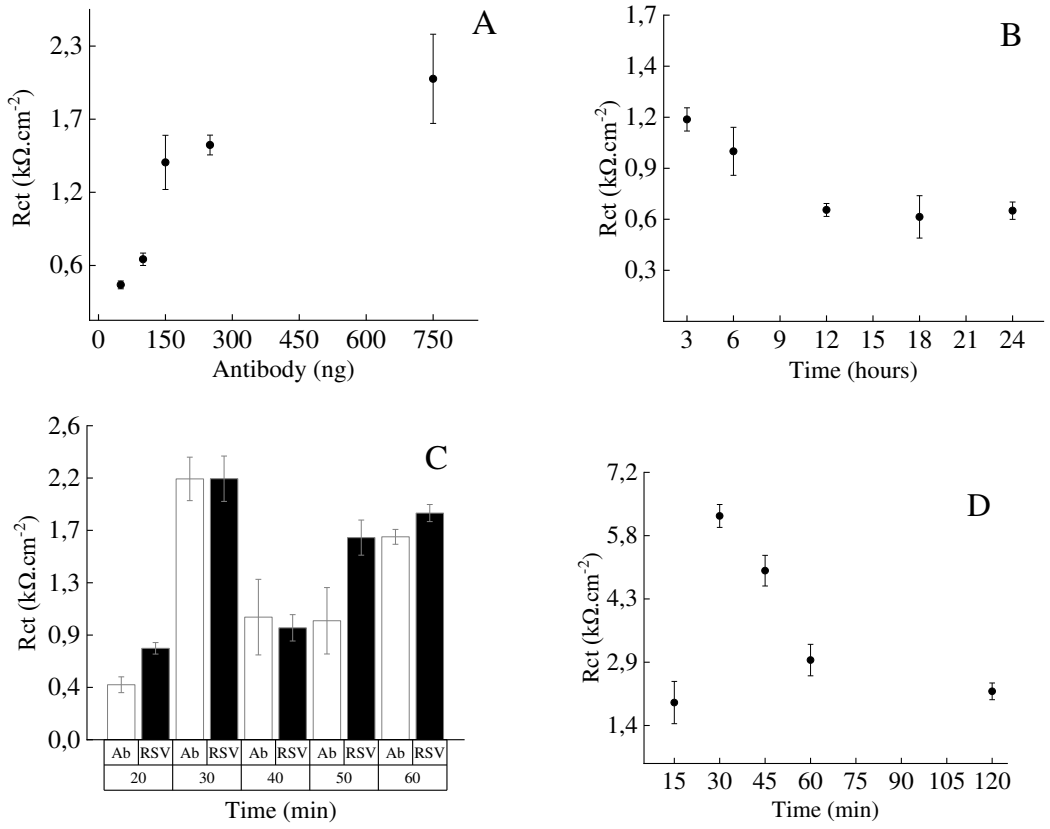
Fig.4. Column chart of R_{ct} obtained through fitting using the Randles circuit from EIS data recorded in $K_3Fe(CN)_6$ (5.0 mM) containing KCl (0.1 M). OCP at 0.25 V; ΔE = 10 mV; Frequency range: 100,000 Hz to 10 mHz. Poly-(3APA)-modified PGE with 30 cycles in a 3APA (2.5 mM) in H_2SO_4 (0.5 M) solution, 50 mV s^{-1}, 100 ng Ab (6.67 ng

μL^{-1}) in PBS 1x pH 7.4 with an incubation time of 3 h, gly = glycine (50.0 mM) in PBS 1x pH 7.4 with an incubation time of 50 min, and VSR ($5.0x10^4$ PFU mL^{-1}) in PBS 1x pH 7.4 with incubation time of 30 minutes.



The biosensor was optimized regarding the amount of antibody, the incubation time with the antibody, glycine, and the virus (**Fig 5**). From **Fig.5A**, it is possible to see that the R_{ct} increase is proportional to the antibody concentration. The two extreme concentration values are not interesting for further analysis. The R_{ct} value for antibody concentration of 50 ng is below the same value obtained for the polymer. At the other end, the R_{ct} for the antibody at 750 ng is somewhat similar to the response obtained at 250 ng. From this sole point of view, one would consider using the 250 ng for further experiments. However, the proper biosensor measurement is based on the differentiation between the Ab-Ag response from the Ab response, and the high Ab concentration might not be suitable from this aspect. Therefore, experiments with Ag must be performed to assess the highest difference between the R_{ct} values. **Fig.S3** presents this data. The value of 250 ng does not provide a good differentiation between the responses. The higher differences come from 100 and 150 ng. We chose 100 ng based on the high reproducibility obtained by comparing the error bars from the two experiments. This choice also results in cost-effectiveness with less expensive biomaterial used, especially considering further mass-scale production for a point-of-care device.

Fig.5. Optimization studies, all starting from PGE/poly-(3APA). All steps were prepared in PBS 1x pH 7.4 solution. R_{ct} obtained through fitting using the Randles circuit from EIS data recorded in $K_3Fe(CN)_6$ (5.0 mM) containing KCl (0.1 M). OCP at 0.25 V; ΔE = 10 mV; Frequency range: 100,000 Hz to 10 mHz. (A) Ab amount with an incubation time of 24 h; (B) Ab incubation time, 100 ng Ab (6.67 ng μL^{-1}); (C) Glycine (50.0 mM) incubation time over PGE/poly-3(APA)/Ab and after immobilization of RSV ($5.0x10^4$ PFU mL^{-1}) with incubation time of 3 hours; and (D) RSV($5x10^4$ PFU mL^{-1}) incubation time.



The R_{ct} response decreases with Ab incubation time (**Fig.5B**), reaching a plateau after 12 hours. Experiments were performed under 3 hours of incubation. However, it was not possible to interpret the results or perform a fitting using the equivalent circuit since the data were all scattered in a non-reproducible way. This condition might be connected with the EDC/NHS presence, which requires more time for the chemical reaction with the biomolecule to occur. It is worth mentioning that the electrodes are plunged into microtubes with the carboxylic acid activation solution and not otherwise (drop-casting the solution over the electrode). The interaction time by plunging is naturally higher than the drop-casting method because of the slow diffusion of the molecules toward the electrode surface. Acceptable Nyquist diagrams were only possible after 3 hours of incubation, with a reproducible response. The R_{ct} values decrease after this time is related

to the saturation of the electrode surface [23]. Therefore, 3 hours were chosen as the Ab incubation time.

The time for glycine incubation was also evaluated as the surface-blocking agent [18,24,25]. The best ΔR_{ct} for glycine incubation time (**Fig.5C**) was 50 minutes. Lastly, the incubation time for the virus was evaluated (**Fig.5D**). It was observed a maximum response at 30 minutes, similar to other reported works regarding respiratory viruses [26,27]. The optimized biosensor was applied in a calibration curve using different RSV concentrations. The linearity was observed from 50.0 and 2000.00 PFU mL^{-1} (**Fig.6**) with a sensitivity of 1.6638 PFU mL^{-1}. The calculated LOD and LOQ were 22.54 and 75.12 PFU mL^{-1}, respectively. The sensor also presents reproducibility with an RSD of 9.38 % (n = 10). **Table 2** contains the data from other published papers regarding the LOD obtained for RSV determination. The proposed biosensor presented lower LOD compared to other electrochemical systems [28,29], similar values compared to the optic sensors [30–34], and specifically to PCR assays [33,35,36]. It is a remarkable feature of a label-free, cost-effective system without any support from the use of nanoparticles.

Fig.6. Calibration curve (50.0 – 2000 PFU mL^{-1}) obtained through EIS for different RSV concentrations recorded in $K_3Fe(CN)_6$ (5.0 mM) containing KCl (0.1 M). OCP at 0.25 V; ΔE = 10 mV; Frequency range: 100,000 Hz to 10 mHz. Poly-(3APA)-modified PGE with 30 cycles in a 3APA (2.5 mM) in H_2SO_4 (0.5 M) solution, 50 mV s^{-1}, 100 ng Ab (6.67 ng μL^{-1}) with an incubation time of 3 h, gly = glycine (50.0 mM) with an incubation time of 50 min, incubation time of RSV of 30 minutes.

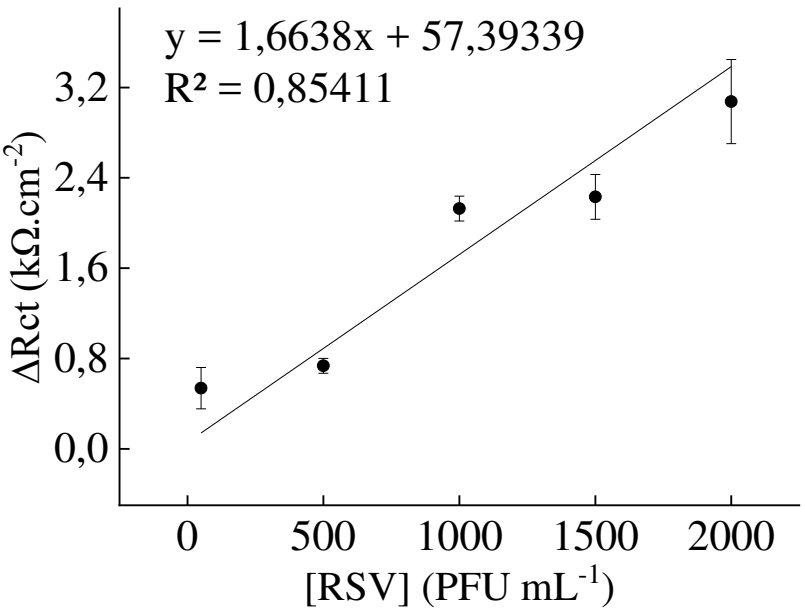


Table 2 – LOD obtained from other different sensors for RSV detection.

Detection method*	LOD (PFU mL^{-1})	Ref
Electrochemical – EIS	1100.00	[28]
Electrochemical – Potentiometric	1000.00	[29]
Optic – Fluorescence	595.00	[30]
Optic – SERS	100.00	[31]
Optic – ELISA	50.00	[32]
Optic – ELISA	16000.00	[33]
Optic – LSPR	40.00	[34]
RTrtPCR	14.13	[35]
TaqMan RT-PCR	1.80-2.30	[36]
RT-qPCR	17.90	[33]
NPA–IPCR	4.10	[33]
Electrochemical – EIS	22.54	This work

*SERS: Surface-enhanced Raman spectroscopy, LSPR: Localized Surface Plasmon Resonance, PCR: Polymerase Chain Reaction, RTrtPCR: real-time Reverse Transcriptase PCR, RT-qPCR: Quantitative Reverse Transcriptase PCR; NPA-PCR: Nanoparticle Amplified Immuno-PCR

Conclusions

A stable polymer derived from 3APA was electrochemically formed and characterized over PGE, resulting in a material with a functionalized carboxyl group. The covalent bond through a nucleophilic acyl substitution reaction with the anti-RSV was effective, and differences in R_{ct} values from EIS data proved suitable for the system evaluation. After proper optimization, the biosensor could detect the RSV obtained through cell culture with good linearity (50.0 to 2000.0 PFU mL^{-1}), low LOD (22.54 PFU mL^{-1}) and high reproducibility (RSD = 9.38). The biosensor performance is outstanding since it applied an ordinary pencil carbon as an electrode, with no aid from nanoparticles, and is label-free through EIS measurements, resulting in values compared to gold standard assays.

Declaration of Competing Interest

The authors report no conflicts of interest.

Acknowledgments

The authors are grateful to the Federal University of Uberlandia for supporting this project. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] H. Jain, J. Schweitzer, N. Justice, StatPearls [Internet], StatPearls Publishing, Treasure Island (FL), 2023. https://www.ncbi.nlm.nih.gov/books/NBK459215/ (accessed November 7, 2023).

[2] T. Shi, D.A. McAllister, K.L. O'Brien, E.A.F. Simoes, S.A. Madhi, B.D. Gessner, F.P. Polack, E. Balsells, S. Acacio, C. Aguayo, I. Alassani, A. Ali, M. Antonio, S. Awasthi, J.O. Awori, E. Azziz-Baumgartner, H.C. Baggett, V.L. Baillie, A. Balmaseda, A. Barahona, S. Basnet, Q. Bassat, W. Basualdo, G. Bigogo, L. Bont, R.F. Breiman, W.A. Brooks, S. Broor, N. Bruce, D. Bruden, P. Buchy, S. Campbell, P. Carosone-Link, M. Chadha, J. Chipeta, M. Chou, W. Clara, C. Cohen, E. de Cuellar, D.A. Dang, B. Dash-yandag, M. Deloria-Knoll, M. Dherani, T. Eap, B.E. Ebruke, M. Echavarria, C.C. de Freitas Lázaro Emediato, R.A. Fasce, D.R. Feikin, L. Feng, A. Gentile, A. Gordon, D. Goswami, S. Goyet, M. Groome, N. Halasa, S. Hirve, N. Homaira, S.R.C. Howie, J. Jara, I. Jroundi, C.B. Kartasasmita, N. Khuri-Bulos, K.L. Kotloff, A. Krishnan, R. Libster, O. Lopez, M.G. Lucero, F. Lucion, S.P. Lupisan, D.N. Marcone, J.P. McCracken, M. Mejia, J.C. Moisi, J.M. Montgomery, D.P. Moore, C. Moraleda, J. Moyes, P. Munywoki, K. Mutyara, M.P. Nicol, D.J. Nokes, P. Nymadawa, M.T. da Costa Oliveira, H. Oshitani, N. Pandey, G. Paranhos-Baccalà, L.N. Phillips, V.S. Picot, M. Rahman, M. Rakoto-Andrianarivelo, Z.A. Rasmussen, B.A. Rath, A. Robinson, C. Romero, G. Russomando, V. Salimi, P. Sawatwong, N. Scheltema, B. Schweiger, J.A.G. Scott, P. Seidenberg, K. Shen, R. Singleton, V. Sotomayor, T.A. Strand, A. Sutanto, M. Sylla, M.D. Tapia, S. Thamthitiwat, E.D. Thomas, R. Tokarz, C. Turner, M. Venter, S. Waicharoen, J. Wang, W. Watthanaworawit, L.M. Yoshida, H. Yu, H.J. Zar, H. Campbell, H. Nair, Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in

2015: a systematic review and modeling study, Lancet. 390 (2017) 946–958. https://doi.org/10.1016/S0140-6736(17)30938-8.

[3] Centers for disease control and prevention, Respir. Syncytial Virus Infect. - RSV Transm. (n.d.). https://www.cdc.gov/rsv/about/transmission.html (accessed November 7, 2023).

[4] The global respiratory syncytial virus burden, Seattle, WA, 2017. https://media.path.org/documents/CVIA_RSV_disease_burden_fs.pdf?_gl=1*d4jirj*_gcl_au*MTU0ODE1NjQwOC4xNjg5MTA2NTY1*_ga*MTk1MzMwNjM1MS4xNjg5MTA2NTY2*_ga_YBSE7ZKDQM*MTY4OTEwNjU2NS4xLjAuMTY4OTEwNjU2NS42MC4wLjA.

[5] J.W. Tang, T.P. Loh, Correlations between climate factors and incidence-a contributor to RSV seasonality, Rev. Med. Virol. 24 (2014) 15–34. https://doi.org/10.1002/rmv.1771.

[6] C. Chartrand, N. Tremblay, C. Renaud, J. Papenburg, Diagnostic accuracy of rapid antigen detection tests for respiratory syncytial virus infection: Systematic review and meta-analysis, J. Clin. Microbiol. 53 (2015) 3738–3749. https://doi.org/10.1128/JCM.01816-15.

[7] T. Popow-Kraupp, J.H. Aberle, Diagnosis of Respiratory Syncytial Virus Infection, Open Microbiol. J. 5 (2011) 128–134. https://doi.org/10.2174/1874285801105010128.

[8] A.T. Borchers, C. Chang, M.E. Gershwin, L.J. Gershwin, Respiratory syncytial virus - A comprehensive review, Clin. Rev. Allergy Immunol. 45 (2013) 331–379. https://doi.org/10.1007/s12016-013-8368-9.

[9] G.R.O. Freitas, D.A.O. Silva, J. Yokosawa, N.T. Paula, L.F. Costa, B.M. Carneiro, L.Z.G. Ribeiro, T.F.M. Oliveira, J.R. Mineo, D.A.O. Queiróz, Antibody response and avidity of respiratory syncytial virus-specific total IgG, IgG1, and IgG3 in young children, J. Med. Virol. 83 (2011) 1826–1833. https://doi.org/10.1002/jmv.22134.

[10] H.S. Magar, R.Y.A. Hassan, A. Mulchandani, Electrochemical impedance spectroscopy (Eis): Principles, construction, and biosensing applications, Sensors. 21 (2021). https://doi.org/10.3390/s21196578.

[11] J. Leva-Bueno, S.A. Peyman, P.A. Millner, A review on impedimetric immunosensors for pathogen and biomarker detection, Med. Microbiol. Immunol. 209 (2020) 343–362. https://doi.org/10.1007/s00430-020-00668-0.

[12] Z. Štukovnik, U. Bren, Recent Developments in Electrochemical-Impedimetric Biosensors for Virus Detection, Int. J. Mol. Sci. 23 (2022). https://doi.org/10.3390/ijms232415922.

[13] H.H. Nguyen, S.H. Lee, U.J. Lee, C.D. Fermin, M. Kim, Immobilized enzymes in biosensor applications, Materials (Basel). 12 (2019) 1–34. https://doi.org/10.3390/ma12010121.

[14] T. Silva, I. Soares, L. Lacerda, T. Cordeiro, L. Ferreira, D. Franco, Electrochemical modification of electrodes with polymers derived from of hydroxybenzoic acid isomers : Optimized platforms for an alkaline phosphatase biosensor for pesticide detection, Mater. Chem. Phys. 252 (2020) 123221. https://doi.org/10.1016/j.matchemphys.2020.123221.

[15] E.B. Aydın, M. Aydın, M.K. Sezgintürk, Fabrication of electrochemical immunosensor based on acid-substituted poly(pyrrole) polymer modified disposable ITO electrode for sensitive detection of CCR4 cancer biomarker in human serum, Talanta. 222 (2021) 121487. https://doi.org/10.1016/j.talanta.2020.121487.

[16] F. Martínez-Rojas, E. Castañeda, F. Armijo, Conducting polymer applied in a label-free electrochemical immunosensor for the detection prostate-specific antigen using its redox response as an analytical signal, J. Electroanal. Chem. 880 (2021). https://doi.org/10.1016/j.jelechem.2020.114877.

[17] M. Beygisangchin, S.A. Rashid, S. Shafie, A.R. Sadrolhosseini, H.N. Lim, Preparations, properties, and applications of polyaniline and polyaniline thin films—a review, Polymers (Basel). 13 (2021). https://doi.org/10.3390/polym13122003.

[18] 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. 117 (2013) 431–437. https://doi.org/10.1016/j.talanta.2013.08.059.

[19] E.M. Geniès, A. Boyle, M. Lapkowski, C. Tsintavis, Polyaniline: A historical survey, Synth. Met. 36 (1990) 139–182. https://doi.org/10.1016/0379-6779(90)90050-U.

[20] F. Alves, I. Castro, D. Leoni, G. Silva, P. De Souza, L. Ricardo, Y. Cristina, D.P. Maia, A. Tatiane, F. Silva, T. Gonçalves, M. Del, P. Taboada, Biosensors and Bioelectronics A novel peptide-based electrochemical biosensor for breast cancer characterization over a poly 3- (3-aminophenyl) propionic acid matrix, Biosens.

Bioelectron. 205 (2022) 114081. https://doi.org/10.1016/j.bios.2022.114081.

[21] P.C.M. Santos, T.M. Lima, P.I. Soares, R.M. Coelho, H.R. Martins, D.B. da Cruz, A.C. Pereira, D.L. Franco, L.F. Ferreira, Electropolymerization in multilayers of aromatic monomers over graphite electrodes for the development of a biosensor for Chagas disease, Mater. Chem. Phys. 288 (2022). https://doi.org/10.1016/j.matchemphys.2022.126364.

[22] Z. Galus, Fundamentals of Electrochemical Analysis, 2nd ed., Polish Scientific Publishers PWN, New York, 1994.

[23] T.S. Svalova, M. V. Medvedeva, A.A. Saigushkina, I. V. Kozitsin, N.N. Malysheva, V.O. Zhdanovskikh, A. V. Okhokhonin, A.N. Kozitsina, A label-free impedimetric immunosensor based on covalent immobilization of anti-E. Coli antibody via a copper-catalyzed azide-alkyne cycloaddition reaction, Anal. Bioanal. Chem. 412 (2020) 5077–5087. https://doi.org/10.1007/s00216-019-02381-1.

[24] P.A.B. Ferreira, M.C.M. Araujo, C.M. Prado, R.A. de Lima, B.A.G. Rodríguez, R.F. Dutra, An ultrasensitive Cystatin C renal failure immunosensor based on a PPy/CNT electrochemical capacitor grafted on interdigitated electrode, Colloids Surfaces B Biointerfaces. 189 (2020) 110834. https://doi.org/10.1016/j.colsurfb.2020.110834.

[25] A.E. Deller, A.L. Soares, J. Volpe, J.G.A. Ruthes, D.E.P. Souto, M. Vidotti, Development of Folate-Group Impedimetric Biosensor Based on Polypyrrole Nanotubes Decorated with Gold Nanoparticles, Biosensors. 12 (2022). https://doi.org/10.3390/bios12110970.

[26] M. Rochelet, S. Solanas, C. Grossiord, P. Maréchal, C. Résa, F. Vienney, C. Barranger, M. Joannes, A thin layer-based amperometric enzyme immunoassay for the rapid and sensitive diagnosis of respiratory syncytial virus infections, Talanta. 100 (2012) 139–144. https://doi.org/10.1016/j.talanta.2012.07.088.

[27] C.L. Wong, M. Chua, H. Mittman, L.X. Choo, H.Q. Lim, M. Olivo, A phase-intensity surface plasmon resonance biosensor for avian influenza a (H5N1) detection, Sensors (Switzerland). 17 (2017) 1–9. https://doi.org/10.3390/s17102363.

[28] W. Białobrzeska, D. Firganek, M. Czerkies, T. Lipniacki, M. Skwarecka, K. Dziąbowska, Z. Cebula, N. Malinowska, D. Bigus, E. Bięga, K. Pyrć, K. Pala, S. Żołędowska, D. Nidzworski, Electrochemical Immunosensors Based on Screen-

Printed Gold and Glassy Carbon Electrodes: Comparison of Performance for Respiratory Syncytial Virus Detection, Biosensors. 10 (2020) 1–13. https://doi.org/10.3390/BIOS10110175.

[29] M.S. Ozdemir, M. Marczak, H. Bohets, K. Bonroy, D. Roymans, L. Stuyver, K. Vanhoutte, M. Pawlak, E. Bakker, A label-free potentiometric sensor principle for the detection of antibody-antigen interactions, Anal. Chem. 85 (2013) 4770–4776. https://doi.org/10.1021/ac400514u.

[30] J.W. Perez, F.R. Haselton, D.W. Wright, Viral detection using DNA functionalized gold filaments, Analyst. 134 (2009) 1548–1553. https://doi.org/10.1039/b904191e.

[31] S. Shanmukh, L. Jones, J. Driskell, Y. Zhao, R. Dluhy, R.A. Tripp, Rapid and Sensitive Detection of Respiratory Virus Molecular Signatures Using a Silver Nanorod Array SERS Substrate, Nano Lett. 6 (2006) 2630–2636. https://doi.org/10.1021/nl061666f.

[32] R.M. Hendry, K. Mcintosh, Enzyme-Linked Immunosorbent Assay for Detection of Respiratory Syncytial Virus Infection: Development and Description, J. Clin. Microbiol. 16 (1982) 324–328.

[33] J.W. Perez, E.A. Vargis, P.K. Russ, F.R. Haselton, D.W. Wright, Detection of respiratory syncytial virus using nanoparticle amplified immuno-polymerase chain reaction, Anal. Biochem. 410 (2011) 141–148. https://doi.org/10.1016/j.ab.2010.11.033.

[34] J. Valdez, S. Bawage, I. Gomez, S.R. Singh, Facile and rapid detection of respiratory syncytial virus using metallic nanoparticles, J. Nanobiotechnology. 14 (2016) 1–12. https://doi.org/10.1186/s12951-016-0167-z.

[35] S.M. Perkins, D.L. Webb, S.A. Torrance, C. El Saleeby, L.M. Harrison, J.A. Aitken, A. Patel, J.P. DeVincenzo, Comparison of a real-time reverse transcriptase PCR assay and a culture technique for quantitative assessment of viral load in children naturally infected with respiratory syncytial virus, J. Clin. Microbiol. 43 (2005) 2356–2362. https://doi.org/10.1128/JCM.43.5.2356-2362.2005.

[36] A. Hu, M. Colella, J.S. Tam, R. Rappaport, S.M. Cheng, Simultaneous detection, subgrouping, and quantitation of respiratory syncytial virus A and B by real-time PCR, J. Clin. Microbiol. 41 (2003) 149–154. https://doi.org/10.1128/JCM.41.1.149-154.2003.

Electronic Supplementary material

Development of a label-free impedimetric immunosensor for the detection of respiratory syncytial virus

Tallita Stéfanne e Silva[a], Guilherme Ramos Oliveira e Freitas[b], Lucas Franco Ferreira[c], Diego Leoni Franco[a*]

Fig.S1. Profile between (A) peak potential *vs* log of current and (B) log of scan rate *vs* peak potential obtained from cyclic voltammograms recorded in 3APA (2.5 mM) in sulfuric acid (0.5 M) over PGE at different scan rates. Inset: equations obtained through linear regression.

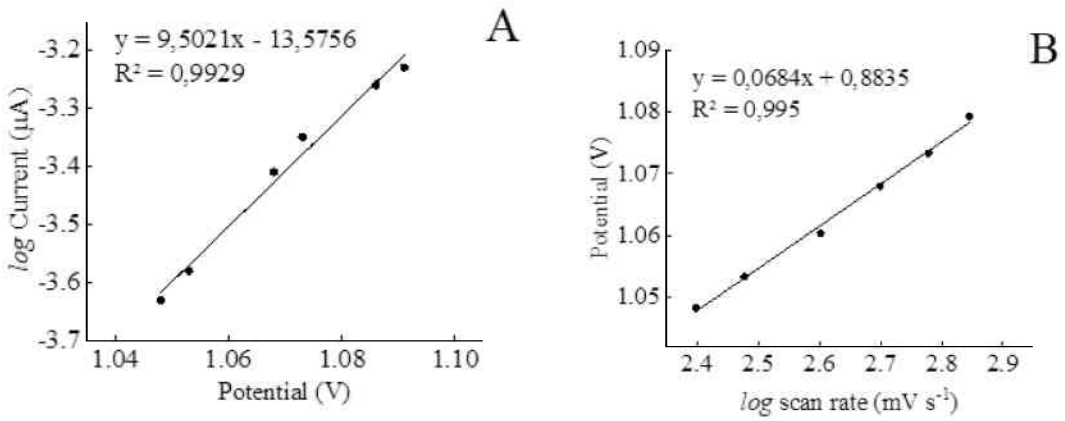


Fig.S2. Profile between peak potential *versus* pH obtained from CVs recorded in 3APA (2.5 mM) in BR buffer (0.04 M) over PCGE, 50 mV s^{-1}. Inset: equation obtained through linear regression.

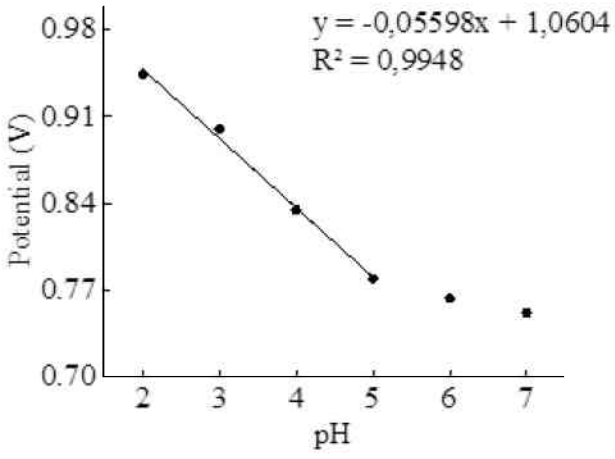
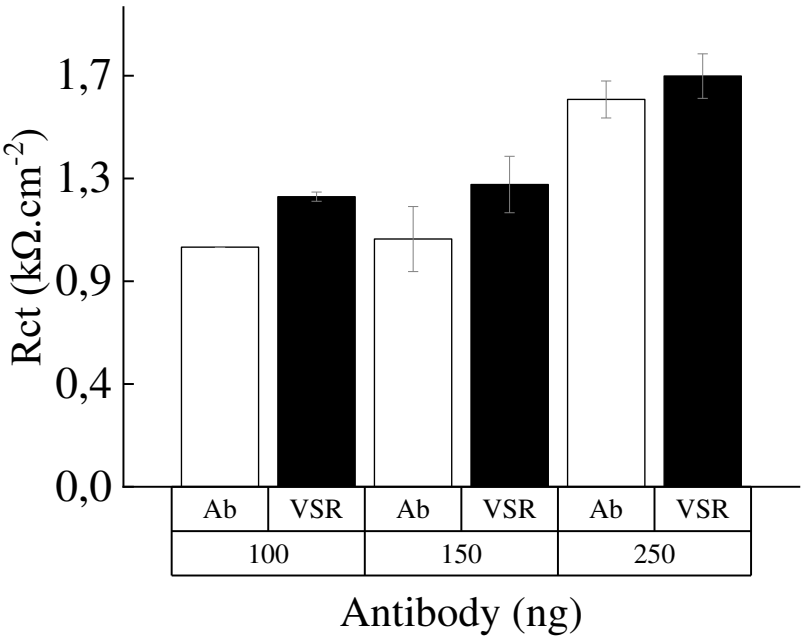


Fig.S3. Column chart of R_{ct} obtained through fitting using the Randles circuit from EIS data recorded in $K_3Fe(CN)_6$ (5.0 mM) containing KCl (0.1 M). OCP at 0.25 V; ΔE = 10 mV; Frequency range: 100,000 Hz to 10 mHz. Poly-(3APA)-modified PGE with 30 cycles in a 3APA (2.5 mM) in H_2SO_4 (0.5 M) solution, 50 mV s^{-1}, 100, 150, and 250 ng Ab (6.67 ng μL^{-1}) in PBS 1x pH 7.4 with an incubation time of 3 h, gly = glycine (50.0 mM) in PBS 1x pH 7.4 with an incubation time of 50 min, and VSR (500 PFU mL^{-1}) in PBS 1x pH 7.4 with incubation time of 3 hours.



3 CONCLUSÃO

Este trabalho mostrou a viabilidade da modificação de PGE com poli(3APA) e a caracterização do material formado através de CV e EIS. As respostas encontradas nos sugerem a formação de um material com a presença de grupos funcionais carboxílicos ativos para ligações covalentes. Os CVs em ácido sulfúrico antes e depois da eletropolimerização indicam que não houve resquício do monômero na superfície do PGE, além de apresentar um par redox após a modificação, indicativo da formação de um material eletroativo.

A otimização do número de varreduras demonstrou que 30 ciclos são suficientes para a modificação completa da superfície do PGE. Os estudos de pH e velocidade, juntamente com as equações de Laviron, Tafel e Nernst possibilitaram propor o mecanismo de eletropolimerização do 3APA, que inicialmente apresenta a perda de um elétron e um próton, gerando um produto com grupos carboxilas livres.

O Ab foi imobilizado de forma efetiva através de ligação covalente no PGE modificado com poli(3APA) e o vírus pode ser detectado por EIS. Foram necessárias 3 h para imobilização do anticorpo e 50 min para o bloqueio da superfície do imunossensor. A detecção de Ag pode ser feita após 30 min de imersão do imunossensor em solução de Ag.

O sistema apresentou um LOD abaixo de valores encontrados na literatura para outros biossensores eletroquímicos, demonstrando ser uma alternativa simples, barata e rápida para a detecção do RSV.

REFERÊNCIAS

ABBAS, A. K.; LITCHTMAN, A. H.; PILLAI, S. Anticorpos e Antígenos. *In*: **Imunologia Celular e Molecular**. 9. ed. São Paulo (BR): Elsevier, 2019a.

ABBAS, A. K.; LITCHTMAN, A. H.; PILLAI, S. Propriedades e Visão Geral das Respostas Imunes. *In*: **Imunologia Celular e Molecular**. 9. ed. Rio de Janeiro: Elsevier, 2019b.

ABBAS, A. K.; LITCHTMAN, A. H.; PILLAI, S. Apêndice III. *In*: **Imunologia Celular e Molecular**. 9. ed. Rio de Janeiro: Elsevier, 2019c.

ABDULBARI, H. A.; BASHEER, E. A. M. Electrochemical Biosensors: Electrode Development, Materials, Design, and Fabrication. **ChemBioEng Reviews**, v. 4, n. 2, p. 92–105, 2017. https://doi.org/10.1002/cben.201600009.

ALBERTS, B.; BRAY, D.; HOPKIN, K.; JOHNSON, A.; LEWIS, J.; RAFF, M.; ROBERTS, K.; WALTER, P. Células: as unidades fundamentais da vida. *In*: **Fundamentos da Biologia Celular**. 4. ed. Porto Alegre: Artmed, 2017a. p. 1–38.

ALBERTS, B.; JOHNSON, A.; LEWIS, J.; MORGAN, D.; RAFF, M.; ROBERTS, K.; WALTER, P.; WILSON, J.; HUNT. Analisando células, moléculas e sistemas. *In*: **Biologia Molecular da Célula**. 6. ed. Porto Alegre: Artmed, 2017b. p. 439–528.

ALBERTS, B.; JOHNSON, A.; LEWIS, J.; MORGAN, D.; RAFF, M.; ROBERTS, K.; WALTER, P.; WILSON, J.; HUNT, T. Proteínas. *In*: **Biologia Molecular da Célula**. 6. ed. Porto Alegre: Artmed, 2017c. p. 109–172.

ALFAYA, A. A. S.; KUBOTA, L. T. A utilização de materiais obtidos pelo processo de sol-gel na construção de biossensores. **Química Nova**, v. 25, n. 5, p. 835–841, 2002. https://doi.org/10.1590/S0100-40422002000500020.

ALMEIDA, N. F.; WINGARD, L. B.; MALMROS, M. K. Immobilization of Glucose Oxidase by Electropolymerization of Monomers: Influence of Polymerization Conditions. **Annals of the New York Academy of Sciences**, v. 613, n. 1, p. 448–451, 1990. https://doi.org/10.1111/j.1749-6632.1990.tb18198.x.

ALVES-BALVEDI, R. P.; CAETANO, L. P.; MADURRO, J. M.; BRITO-MADURRO, A. G. Use of 3,3',5,5' tetramethylbenzidine as new electrochemical indicator of DNA hybridization and its application in genossensor. **Biosensors and Bioelectronics**, v. 85, p. 226–231, 2016. https://doi.org/10.1016/j.bios.2016.05.016.

ALVES, R. da F.; DA SILVA, A. G.; FERREIRA, L. F.; FRANCO, D. L. Synthesis and characterization of a material derived from 4-mercaptobenzoic acid: A novel platform for oligonucleotide immobilization. **Talanta**, v. 165, p. 69–75, 2017. https://doi.org/10.1016/j.talanta.2016.12.025.

BARBOSA, O.; ORTIZ, C.; BERENGUER-MURCIA, Á.; TORRES, R.; RODRIGUES, R. C.; FERNANDEZ-LAFUENTE, R. Glutaraldehyde in bio-catalysts design: A useful crosslinker and a versatile tool in enzyme immobilization. **Royal Society of Chemistry Advances**, v. 4, n. 4, p. 1583–1600, 2014. https://doi.org/10.1039/C3RA45991H.

BASILIO, E.; LAIANE, A.; OLIVEIRA, A. De; VIEIRA, D. S.; FELIPO, L.; SOUZA, B. Infecções Respiratórias de Importância Clínica: uma Revisão Sistemática Review. **Revista Fimca**, v. 4, n. 1, p. 7–16, 2017. https://doi.org/10.37157/fimca.v4i1.5.

BATFLETT, P. N. Modification of sensor surfaces. *In*: SCHULTZ, J. S.; TAYLOR, R. F. **Handbook of Chemical and Biological Sensors1**. 1. ed. Philadelphia (USA): Institute of Physics Publishing, 1996.

BERMAN, S. Epidemiology of acute respiratory infections in children of developing countries. **Reviews of Infectious Diseases**, v. 13, n. Suppl 6, p. S454–S462, 1991. https://doi.org/10.1093/clinids/13.Supplement_6.S454.

BHARDWAJ, T. A Review on Immobilization Techniques of Biosensors. **International Journal of Engineering Research & Technology**, v. 3, n. 5, p. 294–298, 2014.

BORCHERS, A. T.; CHANG, C.; GERSHWIN, M. E.; GERSHWIN, L. J. Respiratory Syncytial Virus - A Comprehensive Review. **Clinical Reviews in Allergy and Immunology**, v. 45, n. 3, p. 331–379, 2013. https://doi.org/10.1007/s12016-013-8368-9.

BOTT, A. W. Electrochemical Techniques for the Characterization of Redox Polymers. **Current Separations**, v. 19, n. 3, p. 71–75, 2001.

BOUSSE, L. Whole cell biosensors. **Sensors and Actuators, B: Chemical**, v. 34, n. 1–3, p. 270–275, 1996. https://doi.org/10.1016/S0925-4005(96)01906-5.

BRUNING, A. H. L.; LEEFLANG, M. M. G.; VOS, J. M. B. W.; SPIJKER, R.; DE JONG, M. D.; WOLTHERS, K. C.; PAJKRT, D. Rapid Tests for Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses: A Systematic Review and Meta-analysis. **Clinical Infectious Diseases**, v. 65, n. 6, p. 1026–1032, 2017. https://doi.org/10.1093/cid/cix461.

BRYCE, J.; BOSCHI-PINTO, C.; SHIBUYA, K.; BLACK, R. E. WHO estimates of the causes of death in children. **Lancet**, v. 365, n. 9465, p. 1147–1152, 2005. https://doi.org/10.1016/S0140-6736(05)71877-8.

CHAI, X.; GAO, J.; CUI, Q.; ZHAO, L. Enzyme-free sandwich-type electrochemical immunosensor based on high catalytic binary PdCu mesoporous metal nanoparticles and conductive black phosphorous nanosheets for ultrasensitive detection of pro-SFTPB in non-small cell lung cancer. **Journal of Electroanalytical Chemistry**, v. 917, n. May, p. 116415, 2022. https://doi.org/10.1016/j.jelechem.2022.116415.

CHANOCK, R.; ROIZMAN, B.; MYERS, R. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA): Isolation, properties and characterization. **American Journal of Epidemiology**, v. 66, n. 3, p. 281–290, 1957. https://doi.org/10.1093/oxfordjournals.aje.a119901.

CLARK, L. C.; LYONS, C. Electrode Systems for Continuous Monitoring in Cardiovascular Surgery. **Annals of the New York Academy of Sciences**, v. 102, n. 1, p. 29–45, 1962. https://doi.org/10.1111/j.1749-6632.1962.tb13623.x.

COLLINS, P. L.; FEARNS, R.; GRAHAM, B. S. Respiratory syncytial virus: Virology, reverse genetics, and pathogenesis of disease. *In*: ANDERSON, L. J.; GRAHAM, B. S. **Challenges and Opportunities for Respiratory Syncytial Virus Vaccines**. Heidelberg: Springer Berlin, 2013. p. 3–38. https://doi.org/10.1007/978-3-642-38919-1_1.

COSTA, L. F.; DA SILVEIRA, H. L.; QUEIRÓZ, D. A. O.; MANTESE, O. C.; YOKOSAWA, J. Respiratory virus infections in hospitalized and non-hospitalized children: Determinants of severe course of the disease. **Journal of Infection in Developing Countries**, v. 16, n. 1, p. 196–205, 2022. https://doi.org/10.3855/jidc.15117.

CRUZ, J. S.; DE SOUZA LUNA, L. K.; ALVES, V. R. G.; CONTE, D. D.; BELLEI, N. C. J. Viral load of respiratory syncytial virus among children from primary care and hospital settings admitted to a university hospital in Brazil (2009-2013). **Journal of Medical Virology**, v. 93, n. 6, p. 3397–3400, 2021. https://doi.org/10.1002/jmv.26185.

DA FONSECA ALVES, R.; FRANCO, D. L.; CORDEIRO, M. T.; DE OLIVEIRA, E. M.; FIREMAN DUTRA, R. A.; DEL PILAR TABOADA SOTOMAYOR, M. Novel electrochemical genosensor for Zika virus based on a poly-(3-amino-4-hydroxybenzoic acid)-modified pencil carbon graphite electrode. **Sensors and Actuators, B: Chemical**, v. 296, n. 2019, 2019. https://doi.org/10.1016/j.snb.2019.126681.

DAMBORSKÝ, P.; ŠVITEL, J.; KATRLÍK, J. Optical biosensors. **Essays in Biochemistry**, v. 60, n. 1, p. 91–100, 2016. https://doi.org/10.1042/EBC20150010.

DHAND, C.; DWIVEDI, N.; MISHRA, S.; SOLANKI, P. R.; MAYANDI, V.; BEUERMAN, R. W.; RAMAKRISHNA, S.; LAKSHMINARAYANAN, R.; MALHOTRA, B. D. Polyaniline-based biosensors. **Nanobiosensors in Disease Diagnosis**, v. 4, p. 25–46, 2015. https://doi.org/10.2147/NDD.S64841.

DONG, S.; ZHAO, R.; ZHU, J.; LU, X.; LI, Y.; QIU, S.; JIA, L.; JIAO, X.; SONG, S.; FAN, C.; HAO, R. Z.; SONG, H. Bin. Electrochemical DNA biosensor based on a tetrahedral nanostructure probe for the detection of avian influenza A (H7N9) virus. **ACS Applied Materials and Interfaces**, v. 7, n. 16, p. 8834–8842, 2015. https://doi.org/10.1021/acsami.5b01438.

GAMELLA, M.; CAMPUZANO, S.; PARRADO, C.; REVIEJO, A. J.; PINGARRÓN, J. M. Microorganisms recognition and quantification by lectin adsorptive affinity impedance. **Talanta**, v. 78, n. 4–5, p. 1303–1309, 2009. https://doi.org/10.1016/j.talanta.2009.01.059.

GIZELI, E.; LOWE, C. R. Immunosensors. **Current Opinion in Biotechnology**, v. 7, n. 1, p. 66–71, 1996. https://doi.org/10.1016/S0958-1669(96)80097-8.

GUAN, J. G.; MIAO, Y. Q.; ZHANG, Q. J. Impedimetric biosensors. **Journal of Bioscience and Bioengineering**, v. 97, n. 4, p. 219–226, 2004. https://doi.org/10.1016/S1389-1723(04)70195-4.

GUILBAULT, G. G.; MOLTAVO JR, J. G. A Urea-Specific Enzyme Electrode. **Journal of the American Chemical Society**, v. 91, n. 8, p. 2164–2165, 1969. https://doi.org/10.1021/ja01036a083.

HARRIS, E. FDA Clears RSV Vaccine for Adults Aged 60 Years or Older. **Medical News in Brief**, v. 329, n. 21, p. 1817, 2023. https://doi.org/10.1001/jama.2023.8657.

HOCK, B. Antibodies for immunosensors - A review. **Analytica Chimica Acta**, v. 347, n. 1–2, p. 177–186, 1997. https://doi.org/10.1016/S0003-2670(97)00167-0.

ICTV. **Human orthopneumovirus**. Disponível em: https://ictv.global/taxonomy/taxondetails?taxnode_id=202101651. Acesso em: 23 out. 2022.

ISLAM, M. N.; MASUD, M. K.; HAQUE, M. H.; HOSSAIN, M. S. Al; YAMAUCHI, Y.; NGUYEN, N.-T.; SHIDDIKY, M. J. A. RNA Biomarkers: Diagnostic and Prognostic Potentials and Recent Developments of Electrochemical Biosensors. **Small Methods**, v. 1, n. 7, p. 1700131, 2017. https://doi.org/10.1002/smtd.201700131.

JAIN, H.; SCHWEITZER, J. W.; JUSTICE, N. A. **Respiratory Syncytial Virus Infection**. Disponível em: https://www.ncbi.nlm.nih.gov/books/NBK459215/#article-28424.s10. Acesso em: 23 out. 2022.

JHA, A.; JARVIS, H.; FRASER, C.; OPENSHAW, P. J. M. Respiratory Syncytial Virus. *In*: HUI, D. S.; ROSSI, G. A.; JOHNSTON, S. L. **SARS, MERS and other Viral Lung Infections**. 1. ed. Sheffield (UK): European Respiratory Society, 2016. p. 1–38.

KHAN, Z. Setup of a PCR Laboratory. *In*: PARK, D. J. **PCR Protocols**. 3. ed. Totowan: Humana Press, 2011. p. 3–14.

KUMAR, D.; SHARMA, R. C. Advances in conductive polymers. **European Pollymer Journal**, v. 34, n. 8, p. 1053–1060, 1998. https://doi.org/10.1016/S0014-3057(97)00204-8.

LABIB, M.; KHAN, N.; GHOBADLOO, S. M.; CHENG, J.; PEZACKI, J. P.; BEREZOVSKI, M. V. Three-mode electrochemical sensing of ultralow MicroRNA levels. **Journal of the American Chemical Society**, v. 135, n. 8, p. 3027–3038, 2013. https://doi.org/10.1021/ja308216z.

LABIB, M.; ZAMAY, A. S.; KOLOVSKAYA, O. S.; RESHETNEVA, I. T.; ZAMAY, G. S.; KIBBEE, R. J.; SATTAR, S. A.; ZAMAY, T. N.; BEREZOVSKI, M. V. Aptamer-based viability impedimetric sensor for bacteria. **Analytical Chemistry**, v. 84, n. 21, p. 8966–8969, 2012a. https://doi.org/10.1021/ac302902s.

LEATHERBARROW, R. J.; EDWARDS, P. R. Analysis of molecular recognition using optical biosensors. **Current Opinion in Chemical Biology**, v. 3, n. 5, p. 544–547, 1999. https://doi.org/10.1016/S1367-5931(99)00006-X.

LEE, S.; JEONG, D.; LEE, S.; NAM, S.; LEE, W.-Y. A Highly Sensitive Amperometric Galactose Biosensor Based on Graphene-doped Sol-gel-derived Titania-Nafion Composite Films. **Electroanalysis**, v. 34, n. 4, p. 629–639, 2021. https://doi.org/10.1002/elan.202100357.

LIU, L.; OZA, S.; HOGAN, D.; CHU, Y.; PERIN, J.; ZHU, J.; LAWN, J. E.; COUSENS, S.; MATHERS, C.; BLACK, R. E. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development

Goals. **The Lancet**, v. 388, n. 10063, p. 3027–3035, 2016. https://doi.org/10.1016/S0140-6736(16)31593-8.

LORENZEN, A. L.; DOS SANTOS, A. M.; DOS SANTOS, L. P.; DA SILVA PINTO, L.; CONCEIÇÃO, F. R.; WOLFART, F. PEDOT-AuNPs-based impedimetric immunosensor for the detection of SARS-CoV-2 antibodies. **Electrochimica Acta**, v. 404, p. 139757, 2022. https://doi.org/10.1016/j.electacta.2021.139757.

MADELEY, C. R.; PEIRIS, J. S. M. Methods in virus diagnosis: Immunofluorescence revisited. **Journal of Clinical Virology**, v. 25, n. 2, p. 121–134, 2002. https://doi.org/10.1016/S1386-6532(02)00039-2.

MADIGAN, M. T.; MARTINKO, J. M.; BENDER, K. S.; BUCKLEY, D. H.; STAHL, D. A. Microrganismos e microbiologia. *In*: **Microbiologia de Brock**. 14. ed. Porto Alegre: Artmed, 2016a. p. 1–24.

MADIGAN, M. T.; MARTINKO, J. M.; BENDER, K. S.; BUCKLEY, D. H.; STAHL, D. A. Microbiologia diagnóstica. *In*: **Microbiologia de Brock**. 14. ed. Porto Alegre: Artmed, 2016b. p. 793–826.

MEDEIROS, E. S.; OLIVEIRA, J. E.; PATERNO, L. G.; MATTOSO, L. H. C. Uso de Polímeros Condutores em Sensores. Parte 1: Introdução aos Polímeros Condutores. **Revista Eletrônica de Materiais e Processos**, v. 7, n. 2, p. 62–77, 2012.

MEHROTRA, P. Biosensors and their applications - A review. **Journal of Oral Biology and Craniofacial Research**, v. 6, n. 2, p. 153–159, 2016. https://doi.org/10.1016/j.jobcr.2015.12.002.

MELERO, J. A. Molecular Biology of Human Respiratory Syncytial Virus. *In*: CANE, P. **Respiratory Syncytial Virus**. 1. ed. Oxford: Elsevier, 2007. p. 1–42. https://doi.org/10.1016/S0168-7069(06)14001-X.

MONTO, A. S. Studies of the community and family: Acute respiratory illness and infection. **Epidemiologic Reviews**, v. 16, n. 2, p. 351–373, 1994. https://doi.org/10.1093/oxfordjournals.epirev.a036158.

MORRIS, J. A.; BLOUNT, R. E.; SAVAGE, R. E. Recovery of Cytopathogenic Agent from Chimpanzees with Goryza. **Proceedings of the Society for Experimental Biology and Medicine**, v. 92, n. 3, p. 544–549, 1956. https://doi.org/10.3181/00379727-92-22538.

MUFSON, M. A.; ORVELL, C.; RAFNAR, B.; NORRBY, E. Two distinct subtypes of human respiratory syncytial virus. **Journal of General Virology**, v. 66, n. 10, p. 2111–2124, 1985. https://doi.org/10.1099/0022-1317-66-10-2111.

NAIR, H.; NOKES, D. J.; GESSNER, B. D.; DHERANI, M.; MADHI, S. A.; SINGLETON, R. J.; O'BRIEN, K. L.; ROCA, A.; WRIGHT, P. F.; BRUCE, N.; CHANDRAN, A.; THEODORATOU, E.; SUTANTO, A.; SEDYANINGSIH, E. R.; NGAMA, M.; MUNYWOKI, P. K.; KARTASASMITA, C.; SIMÕES, E. A.; RUDAN, I.; WEBER, M. W.; CAMPBELL, H. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. **The Lancet**, v. 375,

n. 9725, p. 1545–1555, 2010. https://doi.org/10.1016/S0140-6736(10)60206-1.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **PubChem Compound Summary for CID 571807, 3-Aminophenylacetic acid**. Disponível em: https://pubchem.ncbi.nlm.nih.gov/compound/3-Aminophenylacetic-acid. Acesso em: 6 abr. 2022.

PACHECO, W. F.; SEMAAN, F. S.; DE ALMEIDA, V. G. K.; RITTA, A. G. S. L.; AUCÉLIO, R. Q. Voltametrias: Uma Breve Revisão Sobre os Conceitos. **Revista Virtual de Quimica**, v. 5, n. 4, p. 516–537, 2013. https://doi.org/10.5935/1984-6835.20130040.

PALOMAR, Q.; GONDRAN, C.; MARKS, R.; COSNIER, S.; HOLZINGER, M. Impedimetric quantification of anti-dengue antibodies using functional carbon nanotube deposits validated with blood plasma assays. **Electrochimica Acta**, v. 274, p. 84–90, 2018. https://doi.org/10.1016/j.electacta.2018.04.099.

PATH. **The global respiratory syncytial virus burden**2017. Disponível em: https://media.path.org/documents/CVIA_RSV_disease_burden_fs.pdf. Acesso em 08 abr. 2022.

RALSTON, S. L.; LIEBERTHAL, A. S.; MEISSNER, H. C.; ALVERSON, B. K.; BALEY, J. E.; GADOMSKI, A. M.; JOHNSON, D. W.; LIGHT, M. J.; MARAQA, N. F.; MENDONCA, E. A.; PHELAN, K. J.; ZORC, J. J.; STANKO-LOPP, D.; BROWN, M. A.; NATHANSON, I.; ROSENBLUM, E.; SAYLES, S.; HERNANDEZ-CANCIO, S. Clinical Practice Guideline: The Diagnosis, Management, and Prevention of Bronchiolitis. **American Academy of Pediatrics**, v. 134, n. 5, p. 1474–1502, 2017. https://doi.org/10.1542/peds.2014-2742.

RAMANATHAN, K.; JÖNSSON, B. R.; DANIELSSON, B. Sol-gel based thermal biosensor for glucose. **Analytica Chimica Acta**, v. 427, p. 1–10, 2001. https://doi.org/10.1016/S0003-2670(00)01095-3.

RATH, B.; TIEF, F.; OBERMEIER, P.; TUERK, E.; KARSCH, K.; MUEHLHANS, S.; ADAMOU, E.; DUWE, S.; SCHWEIGER, B. Early detection of influenza A and B infection in infants and children using conventional and fluorescence-based rapid testing. **Journal of Clinical Virology**, v. 55, n. 4, p. 329–333, 2012. https://doi.org/10.1016/j.jcv.2012.08.002.

RICCI, F.; ADORNETTO, G.; PALLESCHI, G. A review of experimental aspects of electrochemical immunosensors. **Electrochimica Acta**, v. 84, p. 74–83, 2012. https://doi.org/10.1016/j.electacta.2012.06.033.

RICHTER, Ł.; BIELEC, K.; LEŚNIEWSKI, A.; ŁOŚ, M.; PACZESNY, J.; HOŁYST, R. Dense Layer of Bacteriophages Ordered in Alternating Electric Field and Immobilized by Surface Chemical Modification as Sensing Element for Bacteria Detection. **ACS Applied Materials and Interfaces**, v. 9, n. 23, p. 19622–19629, 2017. https://doi.org/10.1021/acsami.7b03497.

SCHULTZ, J. S. Biological and chemical components for sensors. *In*: TAYLOR, R. F.; SCHULTZ, J. S. **Handbook of Chemical and Biological Sensors**. 1. ed. Philadelphia: Institute of Physics Publishing, 1996.

SCHULTZ, J. S.; TAYLOR, R. F. Introduction to chemical and biological sensors. *In*: SCHULTZ, J. S.; TAYLOR, R. F. **Handbook of Chemical and Biological Sensors**. 1. ed. Philadelphia: Institute of Physics Publishing, 1996.

SHI, T.; MCALLISTER, D. A.; O'BRIEN, K. L.; SIMOES, E. A. F.; MADHI, S. A.; GESSNER, B. D.; POLACK, F. P.; BALSELLS, E.; ACACIO, S.; AGUAYO, C.; ALASSANI, I.; ALI, A.; ANTONIO, M.; AWASTHI, S.; AWORI, J. O.; AZZIZ-BAUMGARTNER, E.; BAGGETT, H. C.; BAILLIE, V. L.; BALMASEDA, A.; BARAHONA, A.; BASNET, S.; BASSAT, Q.; BASUALDO, W.; BIGOGO, G.; BONT, L.; BREIMAN, R. F.; BROOKS, W. A.; BROOR, S.; BRUCE, N.; BRUDEN, D.; BUCHY, P.; CAMPBELL, S.; CAROSONE-LINK, P.; CHADHA, M.; CHIPETA, J.; CHOU, M.; CLARA, W.; COHEN, C.; DE CUELLAR, E.; DANG, D. A.; DASH-YANDAG, B.; DELORIA-KNOLL, M.; DHERANI, M.; EAP, T.; EBRUKE, B. E.; ECHAVARRIA, M.; DE FREITAS LÁZARO EMEDIATO, C. C.; FASCE, R. A.; FEIKIN, D. R.; FENG, L.; GENTILE, A.; GORDON, A.; GOSWAMI, D.; GOYET, S.; GROOME, M.; HALASA, N.; HIRVE, S.; HOMAIRA, N.; HOWIE, S. R. C.; JARA, J.; JROUNDI, I.; KARTASASMITA, C. B.; KHURI-BULOS, N.; KOTLOFF, K. L.; KRISHNAN, A.; LIBSTER, R.; LOPEZ, O.; LUCERO, M. G.; LUCION, F.; LUPISAN, S. P.; MARCONE, D. N.; MCCRACKEN, J. P.; MEJIA, M.; MOISI, J. C.; MONTGOMERY, J. M.; MOORE, D. P.; MORALEDA, C.; MOYES, J.; MUNYWOKI, P.; MUTYARA, K.; NICOL, M. P.; NOKES, D. J.; NYMADAWA, P.; DA COSTA OLIVEIRA, M. T.; OSHITANI, H.; PANDEY, N.; PARANHOS-BACCALÀ, G.; PHILLIPS, L. N.; PICOT, V. S.; RAHMAN, M.; RAKOTO-ANDRIANARIVELO, M.; RASMUSSEN, Z. A.; RATH, B. A.; ROBINSON, A.; ROMERO, C.; RUSSOMANDO, G.; SALIMI, V.; SAWATWONG, P.; SCHELTEMA, N.; SCHWEIGER, B.; SCOTT, J. A. G.; SEIDENBERG, P.; SHEN, K.; SINGLETON, R.; SOTOMAYOR, V.; STRAND, T. A.; SUTANTO, A.; SYLLA, M.; TAPIA, M. D.; THAMTHITIWAT, S.; THOMAS, E. D.; TOKARZ, R.; TURNER, C.; VENTER, M.; WAICHAROEN, S.; WANG, J.; WATTHANAWORAWIT, W.; YOSHIDA, L. M.; YU, H.; ZAR, H. J.; CAMPBELL, H.; NAIR, H. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. **The Lancet**, v. 390, n. 10098, p. 946–958, 2017. https://doi.org/10.1016/S0140-6736(17)30938-8.

SIMOES, E. A. F.; CHERIAN, T.; CHOW, J.; SHAHID-SALLES, S. A.; LAXMINARAYAN, R.; JOHN, T. J. Acute Respiratory Infections in Children. *In*: **Disease Control Priorities in Developing Countries**. 2. ed. New York: Oxford University Press, 2006. p. 483–497. https://doi.org/10.1596/978-0-8213-6179-5/Chpt-25.

SOARES, I. P.; DA SILVA, A. G.; DA FONSECA ALVES, R.; DE SOUZA CORRÊA, R. A. M.; FERREIRA, L. F.; FRANCO, D. L. Electrochemical enzymatic biosensor for tyramine based on polymeric matrix derived from 4-mercaptophenylacetic acid. **Journal of Solid State Electrochemistry**, v. 23, n. 3, p. 985–995, 2019. https://doi.org/10.1007/s10008-019-04204-w.

SRIVASTAVA, K. R.; AWASTHI, S.; MISHRA, P. K.; SRIVASTAVA, P. K. Biosensors/molecular tools for detection of waterborne pathogens. *In*: PRASAD, M. N. V.; GROBELAK, A. **Waterborne Pathogens: Detection and Treatment**. 1. ed. Oxford: Butterworth-Heinemann, 2020. p. 237–277.

STÉFANNE E SILVA, T.; SOARES, I. P.; GONÇALVES LACERDA, L. R.; REIS CORDEIRO, T. A.; FERREIRA, L. F.; FRANCO, D. L. Electrochemical modification of electrodes with polymers derived from of hydroxybenzoic acid isomers: Optimized platforms for an alkaline phosphatase biosensor for pesticide detection. **Materials Chemistry and Physics**, v. 252, 2020. https://doi.org/10.1016/j.matchemphys.2020.123221.

TAHIR, Z. M.; ALOCILJA, E. C.; GROOMS, D. L. Polyaniline synthesis and its biosensor application. **Biosensors and Bioelectronics**, v. 20, n. 8 SPEC. ISS., p. 1690–1695, 2005. https://doi.org/10.1016/j.bios.2004.08.008.

TANG, J. W.; LOH, T. P. Correlations between climate factors and incidence-a contributor to RSV seasonality. **Reviews in Medical Virology**, v. 24, n. 1, p. 15–34, 2014. https://doi.org/10.1002/rmv.1771.

TAYLOR, G. Immunology of RSV. *In*: CANE, P. **Respiratory Syncytial Virus**. 1. ed. Oxford: Elsevier, 2006. p. 340.

TAYLOR, R. F. Immobilization methods. *In*: SCHULTZ, J. S.; TAYLOR, R. F. **Handbook of Chemical and Biological Sensors**. 1. ed. Philadelphia (USA): Institute of Physics Publishing, 1996.

TAYLOR, R. F.; MARENCHIC, I. G.; SPENCER, R. H. Antibody -and receptor- based biosensors for detection and process control. **Analytica Chimica Acta**, v. 249, n. 1, p. 67–70, 1991. https://doi.org/10.1016/0003-2670(91)87009-V.

THÉVENOT, D. R.; TOTH, K.; DURST, R. A.; WILSON, G. S. Electrochemical biosensors: Recommended definitions and classification. **Biosensors and Bioelectronics**, v. 16, n. 1–2, p. 121–131, 2001. https://doi.org/10.1016/S0956-5663(01)00115-4.

TORRINHA, Á.; AMORIM, C. G.; MONTENEGRO, M. C. B. S. M.; ARAÚJO, A. N. Biosensing based on pencil graphite electrodes. **Talanta**, v. 190, p. 235–247, 2018. https://doi.org/10.1016/j.talanta.2018.07.086.

TUTTLE, R.; WEICK, A.; SCHWARZ, W. S.; CHEN, X.; OBERMEIER, P.; SEEBER, L.; TIEF, F.; MUEHLHANS, S.; KARSCH, K.; PEISER, C.; DUWE, S.; SCHWEIGER, B.; RATH, B. Evaluation of novel second-generation RSV and influenza rapid tests at the point of care. **Diagnostic Microbiology and Infectious Disease**, v. 81, n. 3, p. 171–176, 2015. https://doi.org/10.1016/j.diagmicrobio.2014.11.013.

VÉDRINE, C.; LECLERC, J. C.; DURRIEU, C.; TRAN-MINH, C. Optical whole-cell biosensor using Chlorella vulgaris designed for monitoring herbicides. **Biosensors and Bioelectronics**, v. 18, n. 4, p. 457–463, 2003. https://doi.org/10.1016/S0956-5663(02)00157-4.

VENTURINI ULIANA, C.; YAMANAKA, H. Immunosensor for Detection of the Textile Dye Disperse Orange 1 Based on Non-conventional Competitive Assay. **Electroanalysis**, v. 31, p. 1–7, 2019. https://doi.org/10.1002/elan.201900059.

WANG, B.; ANZAI, J. I. Recent progress in lectin-based biosensors. **Materials**, v. 8, n. 12, p. 8590–8607, 2015. https://doi.org/10.3390/ma8125478.

WANG, J. Electrochemical transduction. *In*: SCHULTZ, J. S.; TAYLOR, R. F. **Handbook of Chemical and Biological Sensors**. 1. ed. Philadelphia (USA): Institute of Physics Publishing, 1996. https://doi.org/10.1201/9781420050486.ch5.

WANG, J. From DNA biosensors to gene chips. **Nucleic Acids Research**, v. 28, n. 16, p. 3011–3016, 2000. https://doi.org/10.1093/nar/28.16.3011.

WANG, S. G.; ZHANG, Q.; WANG, R.; YOON, S. F. A novel multi-walled carbon nanotube-based biosensor for glucose detection. **Biochemical and Biophysical Research Communications**, v. 311, n. 3, p. 572–576, 2003. https://doi.org/10.1016/j.bbrc.2003.10.031.

WATHUO, M.; MEDLEY, G. F.; NOKES, D. J.; MUNYWOKI, P. K. Quantification and determinants of the amount of respiratory syncytial virus (RSV) shed using real time PCR data from a longitudinal household study. **Wellcome Open Research**, v. 1, p. 1–29, 2017. https://doi.org/10.12688/wellcomeopenres.10284.2.

WEN, G.; WEN, X.; SHUANG, S.; CHOI, M. M. F. Whole-cell biosensor for determination of methanol. **Sensors and Actuators, B: Chemical**, v. 201, p. 586–591, 2014. https://doi.org/10.1016/j.snb.2014.04.107.

YEN, C. Y.; WU, W. T.; CHANG, C. Y.; WONG, Y. C.; LAI, C. C.; CHAN, Y. J.; WU, K. G.; HUNG, M. C. Viral etiologies of acute respiratory tract infections among hospitalized children – A comparison between single and multiple viral infections. **Journal of Microbiology, Immunology and Infection**, v. 52, n. 6, p. 902–910, 2019. https://doi.org/10.1016/j.jmii.2019.08.013.

ZHANG, Y.; ZENG, G. M.; TANG, L.; HUANG, D. L.; JIANG, X. Y.; CHEN, Y. N. A hydroquinone biosensor using modified core-shell magnetic nanoparticles supported on carbon paste electrode. **Biosensors and Bioelectronics**, v. 22, n. 9–10, p. 2121–2126, 2007. https://doi.org/10.1016/j.bios.2006.09.030.

ZHOU, X.; LIU, L.; HU, M.; WANG, L.; HU, J. Detection of hepatitis B virus by piezoelectric biosensor. **Journal of Pharmaceutical and Biomedical Analysis**, v. 27, n. 1–2, p. 341–345, 2002. https://doi.org/10.1016/S0731-7085(01)00538-6.

ZUTZ, C.; WAGENER, K.; YANKOVA, D.; EDER, S.; MÖSTL, E.; DRILLICH, M.; RYCHLI, K.; WAGNER, M.; STRAUSS, J. A robust high-throughput fungal biosensor assay for the detection of estrogen activity. **Steroids**, v. 126, n. April, p. 57–65, 2017. https://doi.org/10.1016/j.steroids.2017.07.005.

ANEXO 1 – NORMAS DA REVISTA

TALANTA

The International Journal of Pure and Applied Analytical Chemistry

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0039-9140

DESCRIPTION

Talanta provides a forum for the publication of original research papers, short communications, and critical reviews in all branches of pure and applied **analytical chemistry**. Papers are evaluated based on established guidelines, including the fundamental nature of the study, scientific novelty, substantial improvement or advantage over existing technology or methods, and demonstrated analytical applicability. Original research papers on fundamental studies, and on novel sensor and instrumentation developments, are encouraged. Novel or improved applications in areas such as clinical and biological chemistry, environmental analysis, geochemistry, materials science and engineering, and analytical platforms for omics development are welcome.

Analytical performance of methods should be determined, including interference and matrix effects, and methods should be validated by comparison with a standard method, or analysis of a certified reference material. Simple spiking recoveries may not be sufficient. The developed method should especially comprise information on selectivity, sensitivity, detection limits, accuracy, and reliability. However, *applying official validation or robustness studies to a routine method or technique does not necessarily constitute novelty*. Proper statistical treatment of the data should be provided. Relevant literature should be cited, including related publications by the authors, and authors should discuss how their proposed methodology compares with previously reported methods.

Talanta is the companion title to the open access journal Talanta Open.

The name Talanta derives from the Greek word ταλαντα, used by Homer to mean a *pair of scales*. Such a balance was used for measuring talents or gold coins. See Talanta, 35(5)(1988)I-V for details. The symbol for the journal is a Greek balance taken from one of the Hope Vases.

talantaimage.jpg

AUDIENCE

Analytical Chemists.

IMPACT FACTOR

2021: 6.556 © Clarivate Analytics Journal Citation Reports 20

ABSTRACTING AND INDEXING

Curr Cont ASCA/Phy Chem & Earth Sci
Science Citation Index
Web of Science
Chemical Abstracts
BIOSIS Citation Index
Analytical Abstracts
EIC/Intelligence
Elsevier BIOBASE
Research Alert
Aqualine Abstracts
Scopus

EDITORIAL BOARD

Hian Kee Lee, National University of Singapore, Singapore, Singapore
Miniaturized sample preparation, Separation science, Environmental analysis
Jin-Ming Lin, Tsinghua University, Department of Chemistry, Beijing, China
Microfluidic chip mass spectrometry combined with cell drug metabolism
Baohong Liu, Fudan University, Department of Chemistry, Shanghai, China
Optical/electrochemical biosensing, Microfluidic analysis, Single molecule imaging, Single cell measurement
Frank M. Matysik, University of Regensburg Institute of Analytical Chemistry, Regensburg, Germany
Instumental analytical chemistry, Electroanalysis, Mass spectrometry, Hyphenated systems, Capillary electrophoresis
Maria Minunni, University of Florence, Department of Chemistry 'Ugo Schiff ', Florence, Italy
Chemical Sensors, surface plasmon resonance, Analytical Techniques, Aptamers, PCR, Analytical Chemistry, Lab on a Chip
Alejandro Olivieri, National University of Rosario, Rosario, Argentina
Multivariate analysis, Multiway analysis, Experimental Design, Classification, Calibration.
Sibel A. Ozkan, Ankara University, Department of Analytical Chemistry, Ankara, Turkey
Electroanalysis, Biosensors, Biomarkers, Nanosensors, MIP sensors, chiral sensors
Valérie Pichon, Sorbonne University, Paris, France & ESPCI Paris, PSL University, Paris, France
Sample preparation, Selective extraction, Solid-phase extraction, Immunoaffinity, Aptamers, Imprinted polymers, LC/MS
Feliciano Priego-Capote, University of Cordoba, Department of Analytical Chemistry, Cordoba, Spain
Metabolomics, mass spectrometry, sample preparation, automation, clinical analysis, agrofood analysis
Marcela Segundo, University of Porto, Faculty of Pharmacy, Porto, Portugal
Automation, Miniaturization, Sample treatment
Mojtaba Shamsipur, Razi University, Kermanshah, Iran
Kelly Smalling, US Geological Survey New Jersey Water Science Center, Lawrenceville, New Jersey, United States of America
Petr Solich, Charles University, Department of Analytical Chemistry, Hradec Králové, Czechia
Pharmaceutical analysis, Environmental analysis, Bioanalytical analysis Separation methods (LC, UHPLC), Flow methods (SIA, FIA, SIC) – development and applications Monolithic columns Drugs, Pharmaceuticals
Robert Synovec, University of Washington, Department of Chemistry, Seattle, Washington, United States of America
Comprehensive two-dimensional gas chromatography with mass spectrometry, Applications in fuels, Metabolomics, Foods, Environmental, and Forensics, chemometric data analysis of chromatographic data, high-speed gas chromatography
Beata Walczak, University of Silesia, Katowice, Poland
all aspects of data analysis, chemometrics
Joseph Wang, Arizona State University Biodesign Institute, Tempe, Arizona, United States of America
Amperometric sensors, DNA chips, Glucose monitoring, Microfluidic devices
Xinghua Xia, Nanjing University, Nanjing, China
electrochemical measurements and imaging, in situ infrared spectroelectrochemistry and nano infrared spectroelectrochemical imaging, electrochemical energy conversion and storage, micro-/nano-fluidic devices
Xiu-Ping Yan, Jiangnan University School of Food Science and Technology, Wuxi, China
Food safety, Environmental and biological analysis
Chaoyong Yang, Xiamen University College of Chemistry and Chemical Engineering, Xiamen, China
aptamer, bioluminescence sensor, microfluidic and bioanalysis
Ping Yu, Institute of Chemistry Chinese Academy of Sciences, Beijing, China
Electroanalysis, In vivo analysis, Ion transport, Nanopore, Biosensor
Yong-Liang Yu, Northeastern University, Shenyang, China
Atomic Spectroscopy, Sample pretreatment
Xinrong Zhang, Tsinghua University, Department of Chemistry, Beijing, China
Luminescence analysis, Mass spectrometry

GUIDE FOR AUTHORS

INTRODUCTION

Talanta provides a forum for the publication of original research papers, short communications, and critical reviews in all branches of pure and applied **analytical chemistry**

TYPES OF PAPERS:

Full Papers

Papers are evaluated based on established guidelines, including the fundamental nature of the study, scientific novelty, substantial improvement or advantage over existing technology or methods, and demonstrated analytical applicability. Original research papers on fundamental studies, and on novel sensor and instrumentation developments, are encouraged. Novel or improved applications in areas such as clinical and biological chemistry, environmental analysis, geochemistry, materials science and engineering, and analytical platforms for omics development are welcome.

Short Communications

Short communications comprise more abbreviated studies that demonstrate new concepts and applicability.

Review Articles

Reviews including authoritative and comprehensive review articles, dedicated to a particularly important topic or field of analysis, are published regularly. In addition, shorter, concise reviews or viewpoints focusing on the current status and future prospects of a field or topic particularly relevant to the development of a new analytical methodology or to a better understanding of its fundamental underlying principles are welcome. Tutorial reviews, illustrating in depth fundamental concepts in atomic spectroscopy and analytical atomic spectroscopy, are also published.

CRITERIA:

Analytical performance of methods should be determined, including interference and matrix effects, and methods should be **validated** by comparison with a standard method, or analysis of a certified reference material. Simple spiking recoveries may not be sufficient. The developed method should especially comprise information on selectivity, sensitivity, detection limits, accuracy, and reliability. *However, applying official validation or robustness studies to a routine method or technique does not necessarily constitute novelty*. Proper statistical treatment of the data should be provided. Relevant literature should be cited, including related publications by the authors, and authors should discuss how their proposed methodology compares with previously reported methods.

ESTABLISHED METHODS:

Since classical spectrophotometric measurements and applications (including derivative spectrophometry), fluorimetry, solvent extraction, titrimetry, chemometrics, etc. are well established and are considered routine analytical methods, studies in such areas should demonstrate a unique and substantial advantage over presently known systems. Standard chemometrics for optimization is not novel, but solving a problem or analyzing data may be appropriate. New reagents or systems should demonstrate clear advantage, and their presentation should be comprehensive rather than generating a series of similar papers for several analytes or similar reagents. Modifications of reagents should demonstrate significant improvements. Obvious application of known chemistries or methods to established instrumental techniques are discouraged.

The development of new sensors or methods for common analytes for which many methods exist should demonstrate clear analytical advantage, for example, dopamine, ascorbic acid, uric acid, glucose, H2O2, copper, etc. Nanoparticle based sensors with enhanced sensitivity are now common, and should solve challenging analytical problems.Application of established analytical approaches to specific samples or relatively simple matrices having no major interferences, such as pharmaceutical preparations, are discouraged unless considerable improvements over other methods in the literature are demonstrated, and may be more appropriate for a more focused journal such as a pharmaceutical journal.

DATA:
Papers dealing with analytical data such as stability constants, pKa values, etc. should be submitted to more specific journals, unless novel analytical methodology is demonstrated, or important analytical data are provided which could be useful in the development of analytical procedures.

Submission checklist
You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing
Please see our information on Ethics in publishing.

Declaration of competing interest
Corresponding authors, on behalf of all the authors of a submission, must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. All authors, including those *without* competing interests to declare, should provide the relevant information to the corresponding author (which, where relevant, may specify they have nothing to declare). Corresponding authors should then use this tool to create a shared statement and upload to the submission system at the Attach Files step. **Please do not convert the .docx template to another file type. Author signatures are not required.**

Declaration of generative AI in scientific writing
The below guidance only refers to the writing process, and not to the use of AI tools to analyse and draw insights from data as part of the research process.

Where authors use generative artificial intelligence (AI) and AI-assisted technologies in the writing process, authors should only use these technologies to improve readability and language. Applying the technology should be done with human oversight and control, and authors should carefully review and edit the result, as AI can generate authoritative-sounding output that can be incorrect, incomplete or

biased. AI and AI-assisted technologies should not be listed as an author or co-author, or be cited as an author. Authorship implies responsibilities and tasks that can only be attributed to and performed by humans, as outlined in Elsevier's AI policy for authors.

Authors should disclose in their manuscript the use of AI and AI-assisted technologies in the writing process by following the instructions below. A statement will appear in the published work. Please note that authors are ultimately responsible and accountable for the contents of the work.

Disclosure instructions
Authors must disclose the use of generative AI and AI-assisted technologies in the writing process by adding a statement at the end of their manuscript in the core manuscript file, before the References list. The statement should be placed in a new section entitled 'Declaration of Generative AI and AI-assisted technologies in the writing process'.

Statement: During the preparation of this work the author(s) used [NAME TOOL / SERVICE] in order to [REASON]. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

This declaration does not apply to the use of basic tools for checking grammar, spelling, references etc. If there is nothing to disclose, there is no need to add a statement.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify compliance, your article may be checked by Crossref Similarity Check and other originality or duplicate checking software.

Preprints
Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Preprint posting on SSRN

In support of Open Science, this journal offers its authors a free preprint posting service. Preprints provide early registration and dissemination of your research, which facilitates early citations and collaboration.

During submission to Editorial Manager, you can choose to release your manuscript publicly as a preprint on the preprint server SSRN once it enters peer-review with the journal. Your choice will have no effect on the editorial process or outcome with the journal. Please note that the corresponding author is expected to seek approval from all co-authors before agreeing to release the manuscript publicly on SSRN.

You will be notified via email when your preprint is posted online and a Digital Object Identifier (DOI) is assigned. Your preprint will remain globally available free to read whether the journal accepts or rejects your manuscript.

For more information about posting to SSRN, please consult the SSRN Terms of Use and FAQs.

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer

to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. When coding terminology is used, we recommend to avoid offensive or exclusionary terms such as "master", "slave", "blacklist" and "whitelist". We suggest using alternatives that are more appropriate and (self-) explanatory such as "primary", "secondary", "blocklist" and "allowlist". These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Reporting sex- and gender-based analyses

Reporting guidance

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/ sponsor requirements and best practices within a field. Authors should address the sex and/or gender dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the Sex and Gender Equity in Research (SAGER) guidelines and the SAGER guidelines checklist. These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

Definitions

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous—thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the resources on this page offer further insight around sex and gender in research studies.

Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service
This journal uses the Elsevier Article Transfer Service to find the best home for your manuscript. This means that if an editor feels your manuscript is more suitable for an alternative journal, you might be asked to consider transferring the manuscript to such a journal. The recommendation might be provided by a Journal Editor, a dedicated Scientific Managing Editor, a tool assisted recommendation, or a combination. If you agree, your manuscript will be transferred, though you will have the opportunity to make changes to the manuscript before the submission is complete. Please note that your manuscript will be independently reviewed by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete a 'License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, it is recommended to state this.

Open access

Please visit our Open Access page for more information.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's Author Services.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Please note that a Novelty Statement is required for submission. This statement should provide information in one or two sentences as to what is new and novel in the manuscript in relation to prior related work, including the authors' own.

Please submit your article via https://www.elsevier.com/locate/talanta

Referees

Refereeing of Papers

All papers submitted are refereed by experts who advise the Editors-in-Chief on the matter of acceptance in accordance with the high standards required and on the understanding that the subject matter has not been previously published and that the authors accept full responsibility for the factual accuracy of the data presented and have obtained any necessary authority to publish. Referees will be encouraged to present critical and unbiased reports which are designed to assist the author in presenting material in the clearest and most unequivocal way possible. Authors should appreciate that the comments of referees are presented in a constructive spirit, and that agreement between the views of author and referee must result in a higher standard of publication.

When submitting their paper authors are requested to provide names and addresses (including e-mail addresses) of three competent but independent referees, although the choice of referees used will be made by the Editor.

All papers accepted are subject to editorial amendment if necessary.

PREPARATION

Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our Support Center.

Peer review

This journal operates a single anonymized 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. Editors are not involved in decisions about papers which they have written themselves or have been written by family members or colleagues or which relate to products or services in which the editor has an interest. Any such submission is subject to all of the journal's usual procedures, with peer review handled independently of the relevant editor and their research groups. 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.Page numbering is mandatory for all submitted manuscripts.

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 details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also 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. You can add your name between parentheses in your own script behind the English transliteration. 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. This responsibility includes answering any future queries about Methodology and Materials. **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.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights 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).

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.

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, it is recommended to 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

Follow internationally accepted rules and conventions: use the international system of units (SI). If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUPAC nomenclature for spectroscopy and analytical chemistry for further information (available online at http://www.iupac.org/.). Analytical Terminology is found in the Orange book. IUPAC recommended quantities, units and symbols are found in the Green book.

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.
• Ensure that color images are accessible to all, including those with impaired color vision.

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
Figures and tables should be embedded in text. Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table. All Figures should be accompanied by a caption, e.g.: Fig. 1: Analysis of [...]. (A) Detailed analysis of [...].

Tables
Tables should be placed next to the relevant text in the article. 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.

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.

Reference links
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 highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web Reference
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 should be included in the reference list under a different heading if desired.

Data references
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.

Preprint references
Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

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.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Reference formatting
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. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
[2] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:

[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:
[4] 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:
[5] 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] [6] 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.
Reference to software:
[7] E. Coon, M. Berndt, A. Jan, D. Svyatsky, A. Atchley, E. Kikinzon, D. Harp, G. Manzini, E. Shelef, K. Lipnikov, R. Garimella, C. Xu, D. Moulton, S. Karra, S. Painter, E. Jafarov, S. Molins, Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88), Zenodo, March 25, 2020. https://doi.org/10.5281/zenodo.3727209.

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 file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. 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.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

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.

Research data

This journal requires 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, which may also include 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. When sharing data in one of these ways, you are expected 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 ScienceDirect 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 ScienceDirect.

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).

Research Elements
This journal enables you to publish research objects related to your original research – such as data, methods, protocols, software and hardware – as an additional paper in a Research Elements journal.

Research Elements is a suite of peer-reviewed, open access journals which make your research objects findable, accessible and reusable. Articles place research objects into context by providing detailed descriptions of objects and their application, and linking to the associated original research articles. Research Elements articles can be prepared by you, or by one of your collaborators.

During submission, you will be alerted to the opportunity to prepare and submit a manuscript to one of the Research Elements journals.

More information can be found on the Research Elements page.

Data statement
To foster transparency, we require you to state the availability of your data in your submission if your data is unavailable to access or unsuitable to post. This may also be a requirement of your funding body or institution. You will have the opportunity to provide a data statement during the submission process. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page..

AFTER ACCEPTANCE

Online proof correction
To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.
We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints
The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is

accepted for publication. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.
You can also check the status of your submitted article or find out when your accepted article will be published.