UNIVERSIDADE FEDERAL DE PELOTAS
Centro de Ciências Químicas, Farmacêuticas e de Alimentos
Programa de Pós-Graduação em Bioquímica e Bioprospecção

Tese de Doutorado

Modificações superficiais em pentóxido de nióbio: avaliação estrutural, atividade citotóxica e resposta biológica em *Drosophila melanogaster*

Milena Mattes Cerveira

Pelotas, 2025

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Tese apresentada ao Programa de Pós-Graduação em Bioquímica e Bioprospecção da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciências (área de concentração: Ciências Biológicas II).

Orientador: Rodrigo de Almeida Vaucher

Coorientador: Neftalí Lenin Villareal Carreño

Pelotas, 2025

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação da Publicação

C419m Cerveira, Milena Mattes

Modificações superficiais em pentóxido de nióbio [recurso eletrônico] : avaliação estrutural, atividade citotóxica e resposta biológica em *Drosophila melanogaster* / Milena Mattes Cerveira ; Rodrigo de Almeida Vaucher, orientador ; Neftali Lenin Villarreal Carreno, coorientador. — Pelotas, 2025.
173 f.

Tese (Doutorado) — Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, 2025.

1. Óxidos de nióbio. 2. Stresse oxidativo. 3. *Drosophila melanogaster*. 4. Citotoxicidade. 5. Atividade anticoagulante. I. Vaucher, Rodrigo de Almeida, orient. II. Carreno, Neftali Lenin Villarreal, coorient. III. Título.

CDD 546.524

Elaborada por Ubirajara Buddin Cruz CRB: 10/901

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Tese aprovada como requisito parcial para obtenção do grau de Doutor em Ciências, Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas.

Data da Defesa: 07/03/2025

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Dedico esse trabalho a todos aqueles que, diferente de mim, não puderam priorizar os estudos sobre o trabalho. Também dedico à criança curiosa que já fui e à pesquisadora ainda inquieta que serei sempre: nunca perca a curiosidade de se questionar "por quê?".

Agradecimentos

Gostaria de agradecer, em primeiro lugar, à Universidade Federal de Pelotas, corpo docente e terceirizados: sem todos os profissionais envolvidos, nada disso teria acontecido. À CAPES, pelo fomento da minha bolsa que, sem ela, não seria possível. Um agradecimento especial à Tia Ana e à Pati, por manterem o laboratório sempre limpo e me socorrerem com bicarbonato de sódio quando o meu tinha acabado e o meio de cultura estava pronto.

Ao meu orientador, Rodrigo, por me acolher ainda em 2018 e confiar no meu potencial sem nem saber quem eu era. Agradeço também por todos os ensinamentos, puxões de orelha e, como ele mesmo diz, por "deixar a porta da minha casa aberta, porque somos amigos também". Estendo o agradecimento também à Professora Janice, por compartilhar muitas das minhas dores quando estive viajando, e por sempre ter uma palavra amiga e um direcionamento na pesquisa quando nada parecia fazer sentido. Aos meus colegas do LAPEBBIOM: obrigada por proporcionarem um local engraçado e leve (apesar dos estresses cotidianos), em especial aos que viraram meus amigos e confidentes: meu pupilo Cleiton, Luane, Luize, Patrícia, Thobias, e a tríade de V: Vitor, Victor e Víthor.

Ao meu coorientador Neftalí e ao Professor Cláudio, por acreditarem em mim e me oferecerem o convite do doutorado sanduíche, e por se lembrarem de mim mesmo depois de anos de iniciação científica. Ao Fábio e à Gabriela, pelos ensinamentos, pela paciência em me guiar em uma área completamente diferente e pela amizade construída. Esse trabalho não teria acontecido sem vocês. Um agradecimento ao IC2MP da Université de Poitiers, por me receber durante meu intercâmbio, em especial às minhas orientadoras Catherine, Gwendoline e Laurance, e aos colegas que deixaram o ambiente mais familiar: Frank, Li e Abdennour, e às brasileiras Camila e Lindiane.

À minha família: sou muito grata por ter pessoas tão iluminadas na minha vida e que, mesmo sem entender muito o que eu faço (e o porquê), nunca deixaram de me apoiar, mesmo que isso significasse cruzar um oceano inteiro. Obrigada por entenderem minha ausência, ainda que eu saiba que foi mais dolorido para vocês do que para mim. Em especial aos meus avós Mariza e Ocirio, não só por acreditarem em mim, mas por todo o apoio financeiro dedicado ao longo dos meus estudos para que eu fosse a melhor profissional dentro da minha realidade. Infelizmente, sabemos que muitas vezes esse apoio é indispensável. Aos meus avós Paulo e Lisete, que partiram durante minha graduação, mas que sempre me esperavam com um almoço quentinho quando eu visitava a família. À minha mãe, que, mesmo tendo um relacionamento um pouco diferente da maioria com ela, sempre esteve ali e, por muitos anos, abdicou de tanto para que eu pudesse ter comida na mesa. Podes ter certeza de que, mesmo não sendo de me expressar, eu sempre soube do teu esforço e isso me fez a mulher que sou hoje. Obrigada por sempre me expor a realidade nua e crua como ela é.

Um agradecimento especial à Shaiane, por dividirmos o mesmo neurônio e por sempre ter certeza de que eu estava me alimentando bem (quase nunca) e por a gente “sempre dar um jeito”; à Karline, pela amizade repentina e por nossas conversas serem um “espaço de terapia” no final do doutorado de ambas, além de muitos memes e risadas; à Isadora e ao Ryan, pelo companheirismo durante o voluntariado nas enchentes, pela amizade criada, e por estarem sempre dispostos a um abraço, mesmo eu não gostando de abraços. Gostaria de agradecer à Taciane, pela parceria que surgiu no mestrado no meio da pandemia e que se tornou uma amizade próxima, e por sempre estar disposta a ouvir minhas peripécias; e à Inês, pela leveza da amizade, e por me ajudar a aproveitar os momentos de relaxamento nessa reta final (mesmo que a cabeça não saiba parar às vezes).

Gostaria de agradecer, mesmo que ausente na minha vida, à Camila, por me encorajar a voltar para o doutorado, quando eu precisei desistir por não haver fomento. Um agradecimento especial a todas as pessoas que conheci em Poitiers, excepcionalmente à Luana, que me abraçou como parte da família e sempre teve uma palavra para me ajudar no que fosse preciso, e ao Antoine, por trazer leveza à minha caminhada.

Agradecer também às academias e profissionais delas, principalmente ao Arthur, Douglas e Thiago. Ainda que um agradecimento incomum, ter um momento para desopilar com algo que se gosta de fazer deveria ser menos negligenciado na pós-graduação. Ao Lu e ao Beto do Boteco Skina, por sempre me receberem como se fosse minha segunda casa, com uma cerveja gelada e uma boa conversa para desligar a mente no final do dia. À minha cachorra Manteiga, que mesmo não entendendo nada, me fez companhia em muitas noites viradas e me obrigava a sair

de casa para pegar sol quando eu passava o dia no computador, e à Luna, nessa reta final, que parecia saber quando eu estava esgotada e sempre me trazia um brinquedo todo babado para me alegrar.

Um agradecimento especial a todos que também não mencionei, mas que se fizeram presentes em diferentes etapas do doutorado. Fica difícil citar todos, mas saibam que cada um que passou teve sua importância, ainda que todos os nomes não apareçam aqui.

E por fim, mas não menos importante, agradecer a mim mesma por sempre querer ser melhor e nunca deixar de aprender. Agradeço também por nunca ter desistido, embora muitas vezes durante toda a minha trajetória eu tenha me questionado sobre minhas escolhas e minha capacidade. Quero agradecer, finalmente, por ter segurado firme quando eu mesma quis naufragar.

O trabalho pode ter meu nome como principal autora, mas eu não teria chegado até aqui sozinha, física e mentalmente. Tenham certeza de que esse caminho foi muito mais leve por causa de vocês.

Cada parte de mim é feita de uma parte de vocês.

“Se você quiser fazer uma torta de maçã a partir do zero,

você deve primeiro inventar o Universo.”

Carl Sagan

Resumo

CERVEIRA, Milena Mattes. **Modificações superficiais em pentóxido de nióbio: avaliação estrutural, atividade citotóxica e resposta biológica em *Drosophila melanogaster*.** 2025. 199f. Tese de Doutorado - Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, 2025.

A busca por novos biomateriais tem impulsionado o estudo de óxidos metálicos, como o óxido de nióbio (Nb_2O_5), devido à sua resistência à corrosão, inércia química e relevância econômica para o Brasil. O Nb_2O_5 apresenta aplicações interessantes na área da catálise revestimentos metálicos, vidros e polímeros, demonstrando a modulação a proliferação e adesão celular, bem como a redução dos níveis de marcadores inflamatórios. No entanto, há uma escassez de estudos sobre suas modificações superficiais e interações biológicas. Este estudo buscou caracterizar físico-quimicamente e avaliar a citotoxicidade in vitro e a toxicidade in vivo de óxidos de nióbio modificados superficialmente utilizando diferentes surfactantes, sendo eles ácido cítrico, ácido oleico, dodecil sulfato de sódio (SDS) e brometo de cetrimônio. As amostras de Nb_2O_5 foram avaliadas utilizando técnicas infravermelho por transformada de Fourier (FTIR), espectroscopia RAMAN e difração de raios-x (DRX), mas não foi detectada nenhuma adição de grupos funcionais provindas dos surfactantes. O Nb_2O_5 puro obteve a maior área superficial (230,4 mg^2/g) através da técnica de Brunauer-Emmett-Teller, e o Nb_2O_5 modificado com 0,5 mmol de SDS (NbSDS5) apresentou a maior acidez (3141 μmol/g), a partir da avaliação por dessorção de amônia. Por meio de análises *in vitro* e *in vivo*, observou-se um comportamento dose-dependente para a atividade hemolítica, variando de 1 a 10 mg/ml, sendo Nb_2O_5 puro altamente citotóxico para os eritrócitos (85% morte celular), e a modulação da via intrínseca de coagulação. Ademais, a citotoxicidade demonstrou proliferação celular para Nb_2O_5 modificado com 0,1 mmol de SDS (NbSDS1) mesmo em condições de pH baixo, mas aparentemente sem correlação com o extravasamento de íons de Nb para o meio. A exposição por via oral utilizando *Drosophila melanogaster* (10x e 50x a IC_{50} da atividade hemolítica) demonstrou que, mesmo a administração aguda (72h) foi capaz de alterar parâmetros como glicose, tiois não proteicos (NPSH) e espécies reativas. Entretanto, não modulou os níveis de triglicerídeos e peroxidação lipídica, podendo indicar um efeito protetor do tecido adiposo, uma vez que os níveis de NPSH aumentaram. Estes estudos demonstram, pela primeira vez, a exposição do Nb_2O_5 em eritrócitos, hepatócitos, via de coagulação e em um modelo animal de *D. melanogaster*.

Palavras-chave: óxidos de nióbio; estresse oxidativo; *Drosophila melanogaster*; citotoxicidade; atividade anticoagulante

Abstract

CERVEIRA, Milena Mattes. **Surface Modifications in Niobium Pentoxide: Structural Evaluation, Cytotoxic Activity, and Biological Response in *Drosophila melanogaster*.** 2025. 199p. Thesis (Doctorate) - Programa de Pós-Graduação em Bioquímica e Bioprospeccão, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, 2025.

The search for novel biomaterials has triggered the investigation of metal oxides, particularly niobium oxide (Nb_2O_5), given its corrosion resistance, chemical inertness, and economic importance to Brazil. Nb_2O_5 possesses great applications in catalysis, metallic coatings, glass, and polymers, exhibiting an alteration on cell proliferation and adhesion, with a decrease in inflammatory marker levels. However, there is an absence of research about its surface modifications and biological interactions. This study aimed to physicochemically characterize and evaluate the in vitro cytotoxicity and in vivo toxicity of surface-modified niobium oxides using different surfactants, namely citric acid, oleic acid, sodium dodecyl sulfate (SDS), and cetrimonium bromide. The Nb2O5 samples were analyzed using Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, and X-ray diffraction (XRD), but no addition of functional groups from the surfactants was detected. Pure Nb_2O_5 exhibited the highest surface area (230.4 m²/g) using the Brunauer-Emmett-Teller technique, while Nb_2O_5 modified with 0.5 mmol of SDS (NbSDS5) showed the highest acidity (3141 µmol/g), as assessed by ammonia desorption. Through in vitro and in vivo analyses, a dose-dependent behavior was observed for hemolytic activity, ranging from 1 to 10 mg/mL, with pure Nb2O5 being highly cytotoxic to erythrocytes (85% cell death) and modulating the intrinsic coagulation pathway. Furthermore, cytotoxicity assays indicated cell proliferation for Nb_2O_5modified with 0.1 mmol of SDS (NbSDS1), even under low pH conditions, but apparently without correlation to the release of Nb ions into the medium. Oral exposure using Drosophila melanogaster (10× and 50× the IC50 of hemolytic activity) demonstrated that even acute administration (72 h) was capable of altering parameters such as glucose, non-protein thiols (NPSH), and reactive species. However, it did not modulate triglyceride levels or lipid peroxidation, which may indicate a protective effect of adipose tissue, as NPSH levels increased. These studies demonstrate, for the first time, the exposure of $Nb_2O_5$to erythrocytes, hepatocytes, the coagulation pathway, and an animal model of D. melanogaster.

Keywords: niobium oxide, oxidative stress, Drosophila melanogaster, cytotoxicity, anticoagulant activity.

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Sumário

1 INTRODUÇÃO

Com o crescimento da área biomédica/farmacêutica e a necessidade de se buscar novos materiais que sirvam para a área protética, *drug delivery*, e desenvolvimento de equipamentos médicos, os óxidos metálicos têm sido alvos de diversos estudos (Safavi; Khalil-Allafi; Visai, 2023). A utilização do óxido de Nióbio (Nb_2O_5) vem crescendo em estudos na área biológica, por sua resistente corrosão, inércia química e por injetar o Brasil no mercado econômico, uma vez que o país é o principal minerador de Nb (Eduok, 2020; Gałaś; Krzak; Szlugaj, 2024)

O Nb_2O_5 possui um amplo espectro de aplicações como revestimento de materiais metálicos (Safavi; Khalil-Allafi; Visai, 2023) vidros (Madhavi *et al.*, 2021), e polímeros (Marins *et al.*, 2019). Alguns materiais demonstraram causar proliferação celular *in vitro* em células de cementoblastomas (Ramírez *et al.*, 2011) e melhor adesão celular (Amaravathy *et al.*, 2014). Em parâmetros inflamatórios, o Nb_2O_5 diminuiu fatores como o TNF- α e a IL-17 em um material de ácido inoxidável revestido com Nb_2O_5 (Moreto *et al.*, 2021). No entanto, a literatura é muito escassa quanto à modificação superficial do próprio ON e da interação biológica deste.

Existem diversas estratégias de modificação superficial que visam a otimização de possíveis biomateriais, como a adição de ZnO em vidros e polímeros, melhorando a afinidade com a água (Singh; Singh, 2018). A modificação de vidros com a presença de Nb_2O_5 pode melhorar a atividade antibacteriana contra *Staphyloccus aureus* (madhavi). O uso em adesivos ortodônticos também se mostrou promissor, ao reduzir a quantidade de colônias de *Strepcoccus mutants* (UA 159) (Altmann *et al.*, 2017)

De modo a avaliar a eficiência na modificação superficial, são necessárias abordagens envolvendo estudos *in vitro* e *in vivo*, permitindo a análise de parâmetros em condições controladas, como viabilidade celular e modulação de marcadores bioquímicos. Nesse contexto, a utilização de linhagens celulares importantes no transporte de oxigênio, como os eritrócitos, e células responsáveis pela detoxificação de xenobióticos, como os hepatóticos, são modelos interessantes para uma triagem inicial. Ademais, o uso do inseto *Drosophila melanogaster* vem se mostrando promissor para avaliações de marcadores bioquímicos e modulação da expressão gênica, pela alta capacidade de reprodução, baixo custo e similaridade com o genoma humano, além de diminuir o uso de modelos mamíferos (Dutta *et al.*, 2021; Pagano; Faggio, 2015)

Apesar do crescente interesse na utilização do Nb_2O_5 na área biomédica, ainda existem lacunas quanto ao entendimento do comportamento do Nb_2O_5 no organismo. Compreender essas interações é essencial para a validação da segurança de um biomaterial. Diante disso, o presente estudo tem como objetivo analisar os efeitos de modificações superficiais em Nb_2O_5 através de análises *in vivo* e *in vitro*, com ênfase na caracterização destes materiais, bem como na avaliação de citotoxicidade e modulação do metabolismo de *D. melanogaster*. Os resultados contribuirão para o conhecimento sobre a biocompatibilidade do Nb_2O_5.

2 OBJETIVOS

2.1 Objetivos gerais

Caracterizar físico-quimicamente e avaliar a citotoxicidade *in vitro* e a toxicidade *in vivo* de pentóxido de nióbio modificados superficialmente.

2.2 Objetivos específicos

- Caracterizar morfologicamente os óxidos a base de Nióbio;
- Qualificar os sítios ácidos dos materiais;
- Estudar o comportamento cristalino;
- Descrever a composição molecular;
- Avaliar a citotoxicidade dos compostos em células hepáticas de Zebrafish (ZF-L);
- Examinar a atividade hemolítica e anticoagulante dos óxidos a base de Nióbio;
- Estudar a segurança e toxicidade dos compostos em *D. melanogaster;*
- Explorar a atividade antioxidante dos compostos de nióbio em *D. melanogaster*;

3 REVISÃO DE LITERATURA

3.1 Nióbio

3.1.1 História e propriedades

O nióbio (Nb) é um metal de transição localizado na segunda posição da quinta coluna da tabela periódica. Este composto foi inicialmente referido como "*Columbium*" por Charles Hachett em 1802 (Hachett, 1832), nome que faz referência à Columbia, um nome antigo para os Estados Unidos da América. Posteriormente, recebeu o nome de *Niobium*, como homenagem à Niobe, deusa mitológica e filha de Tantalus e, nos anos de 1950, a *International Union of Pure and Applied Chemistry* (IUPAC) oficializou o nome Nióbio (Rayner-Canham; Zheng, 2008). Por muito tempo, foi confundido com o tântalo, por apresentarem características físicas e químicas similares.

Quimicamente, possui número atômico 41, massa atômica 92,9 u e não é encontrado de forma livre na natureza, mas sim em associação com minerais. Seus pontos de fusão e ebulição são 2477 ^{o}C e 4744 ^{o}C, respectivamente (Nico; Monteiro; Graça, 2016). Possui uma ampla gama de estados de oxidação, partindo do -1 ao +5, sendo este último o mais estável. A Tabela 1 apresenta as propriedades do Nb de forma resumida. Estruturalmente, o Nb é considerado um cristal cúbico de corpo centrado (Figura 1), similar à estrutura do cloreto de sódio (NaCl). É um metal altamente resistente à corrosão e, em sua forma pura, é macio, de coloração cinza, com alta condutividade e maleabilidade. Ainda que maleável, o Nb, quando adicionado a ligas de Ferro (Fe) ou Níquel (Ni), aumenta a dureza das mesmas, sendo bastante utilizado na construção civil (52,4%), automotiva (29%), energia (11,4%) e engenharia aeroespacial (3%) (Gałaś; Krzak; Szlugaj, 2024b).

Tabela 1: Propriedades físicas e químicas do nióbio.

Propriedade	Valor
Número atômico	41
Massa atômica	92.906 u
Ponto de fusão	2447 ^{o}C
Ponto de ebulição	4741 ^{o}C
Densidade	8.57 g/cm3
Estados de oxidação	- I, 0, + I, + II, + III, + IV, + V

Além disso, o Nb consegue diminuir a pegada de carbono (Companhia Brasileira de Metalurgia e Mineração, [s.d]). Dentre suas aplicações, pode-se citar a produção de superligas, capacitores, baterias, vidros etc. Mais informações sobre a aplicação em áreas específicas podem ser encontradas nos estudos (Benedito *et al.*, 2024; Satya Prasad; Baligidad; Gokhale, 2017; Zhou *et al.*, 2020), entre outros.

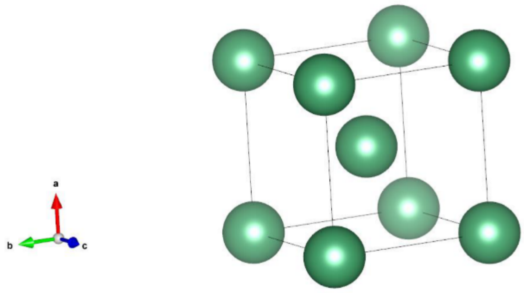

Figura 1: Estrutura cristalina do Nb.

O geólogo brasileiro Djalma Guimarães descobriu em Araxá, Minas Gerais, no século XX, a maior mina de pirocloro (um óxido raro na natureza) e, consequentemente, de Nb, mundialmente. O pirocloro consiste na fórmula química ($A_2Nb_2(O, OH)_6Z$), onde A = Na, Ca, Sn^{2+}, Sr, Pb^{2+} Sb^{3+}, Y, U^{4+}, H_2O ou vacância, e Z = OH, F, O, H_2O ou uma vacância. A Companhia de Desenvolvimento Econômico de Minas Gerais (2021) elucida que a maior prevalência de Nb na natureza provém da columbita-tantalita $(Fe,Mn)(Nb,Ta)_2O_6$, com teor máximo de 76% de óxido de nióbio (Nb_2O_5), do pirocloro $(Na_2,Ca)_2(Nb,Ti)(O,F)_7$ (teor máximo de 71%) e da loparita $(Ce,Na,Ca)_2(Ti,Nb)_2O_6$ (máximo de 20%).

3.1.2 Mercado econômico

Economicamente, o mercado do Nb é considerado um oligopólio, ou seja, há apenas um pequeno número de empresas responsáveis pela produção e/ou comercialização (Dicionário Online Priberam de Português, [*s. d.*])). Existem três empresas principais que possuem aproximadamente 98% da distribuição mundial: Companhia Brasileira de Metalurgia e Mineração (CBMM), CMOC Group Limited e Niobec, do grupo Magris Performance Materials (Gałaś; Krzak; Szlugaj, 2024). A

CBMM, sozinha, ocupa cerca de 78% do mercado, sendo líder em produção, preço e vendas. A produção de Nb no Brasil em 2023 foi de aproximadamente 75.000 toneladas, representando cerca de 90% do mercado, seguido do Canadá (segundo maior país exportador de Nb), com apenas 7 mil toneladas (8,4%). Segundo Serviço Geológico Brasileiro, o Nb é o terceiro maior mineral exportado do Brasil, atrás apenas de Ferro e Ouro. Os valores de mercado do Nb, de acordo com o levantamento de (Bakry, Li e Zeng, 2022), giram em torno de USD 42,0 por quilograma (kg), enquanto a aquisição de FeNb é de USD 46 por kg. A diferença não é tão discrepante uma vez que a liga FeNb ocupa mais de 90% do mercado do Nb.

De acordo com o Ministério de Minas e Energia, houve um crescimento de 30,4% em 2021 na produção mundial de Nb, sendo 92,5% do mercado exclusivamente do Brasil (totalizando quase 110 mil toneladas). O produto mais comercializado ainda é a liga FeNb, sendo a China o maior importador do Brasil, com cerca de 34% de material importado. Dada a abundante disponibilidade de Nb em território brasileiro, e a majoritariedade do oligopólio, é evidente a necessidade de explorar a mineração e descobrir novos compostos e aplicações, de forma a injetar ainda mais o Brasil no mercado internacional.

O Governo Brasileiro, por intermédio de dois programas, provindos do Ministério da Ciência, Tecnologia e Inovações (MCTI), denominados de Projeto Nióbio, dos anos 1970, idealizado pelo então Secretário de Tecnologia Industrial do MCTI, Dr. José Walter Bautista Vidal, durante o governo ditatorial de Ernesto Geisel, e o Programa InovaNióbio, de 2022, no governo de Jair Bolsonaro, estabeleceram a promoção da pesquisa em torno do Nb. Ambos os projetos visavam a promoção da inovação do Nb para a produção de óxidos, ligas e metais, para diversas áreas do conhecimento (Portaria MCTI, 6.002/22).

O Instituto Alberto Luiz Coimbra de Pós-Graduação e Pesquisa de Engenharia (COPPE) da Universidade Federal do Rio de Janeiro (UFRJ) patenteou internacionalmente em 2009 uma tinta anticorrosiva à base de Nb, prometendo ser uma alternativa eficaz e de melhor custo-benefício que o aço inoxidável (Luiz Roberto Miranda, Ladimir José Carvalho, Antônio Carlos Pereira, 2001). O Nb é um metal altamente resistente à corrosão, pois suas mudanças de fases acontecem em temperaturas elevadas, acima de 500 °C. Assim, o revestimento de Nb (chamado de niobização) atuaria como uma proteção em ambientes altamente corrosivos, em indústrias que lidam com gases corrosivos, substâncias submetidas a altas temperaturas ou reagentes químicos, como petro e termoquímicas. Infelizmente, não foram encontrados dados mais recentes que demonstrem a aplicabilidade atual destes programas no ano em questão.

O Fluxograma 1 apresenta a linha cronológica do processo de produção do Nb, desde a sua mineração, sendo o pirocloro o mineral mais utilizado, até sua reciclagem ou descarte. De acordo com Alves e Coutinho (2019), o gerenciamento de resíduos de Nb é classificado pela Norma Brasileira (NBR) 10.004, incluindo a classificação de sólidos gerados durante o processo de minério e concentração/refinamento. Os resíduos são caracterizados com base nas suas características física e química e potencial impacto ambiental, tais como resíduos sólidos (partículas finas, particulados, areias), líquidos (efluentes de emissões gasosas e industriais), gasosos (poluentes aéreos) e radioativos (que podem conter urânio e tório). Os métodos de reciclagem do Nb envolvem processos hidro metalúrgicos, como a lixiviação ácida envolvendo ácido hidro fluorídrico e ácido nítrico, e refinamento eletroquímico, com a dissolução de Nb em sais fundidos, que permite a degradação de produtos em Nb em pó de alta pureza (Lyashok, Brovin e Tashlykovych, 2016).

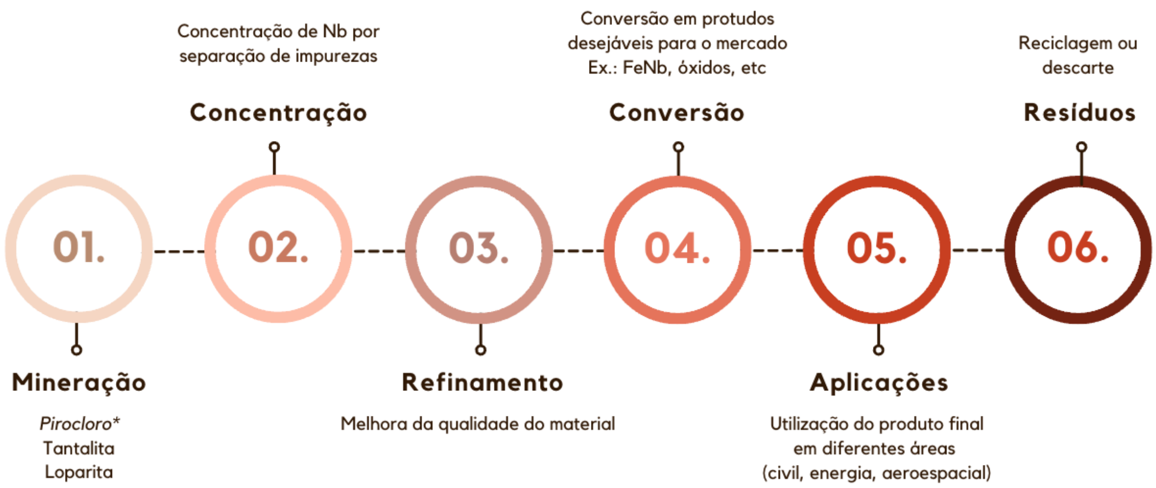

Figura 2: Fluxograma do processo produtivo do Nb.

3.1.3 Óxido de nióbio

O Nb ainda apresenta grande afinidade pelo oxigênio, desencadeando a produção de diferentes óxidos. O oxigênio permite que ocorra a alteração das propriedades desse metal, como a alteração de estados de oxidação, podendo adquirir configuração +II, +IV e +V. A interação desse gás é proporcional ao aumento da temperatura e, ao passo que se aumenta a resistividade do Nb, diminui a temperatura de trabalho como semicondutor. Os óxidos principais formados pela reação entre os dois elementos são o monóxido de nióbio (NbO), dióxido de nióbio (NbO_2) e o pentóxido de nióbio (Nb_2O_5), este último sendo o mais estável termodinamicamente e amplamente pesquisado para diferentes propósitos comerciais (Figura 3). Normalmente é também chamado apenas de óxido de nióbio (Nb_2O_5) (García-López et al., 2024; Nico; Monteiro; Graça, 2016; Valencia-Balvín et al., 2014).

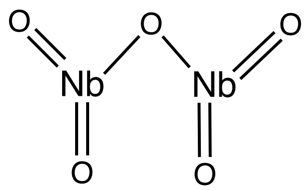

Figura 3: Estrutura química do óxido de nióbio.

Fisicamente, o Nb_2O_5 é um pó branco e, a altas temperaturas, pode adquirir uma coloração amarela. É quimicamente estável na presença de ar e insolúvel na maioria dos reagentes, sendo atacado majoritariamente por ácidos fortes, como o ácido fluorídrico (HF). Apresenta estrutura eletrônica [Kr]4d0, onde os elétrons 4d interagem com o oxigênio na banda 2p e um band gap de 3.1-4.1 eV (Beknalkar et al., 2025). Essa configuração dá ao Nb_2O_5 uma condutividade elétrica menor quando comparada aos outros óxidos, pois é o mais estável dentre eles e, consequentemente, apresenta menos elétrons livres. O Nb_2O_5 apresenta também um grande polimorfismo, ou seja, a formação de diferentes fases cristalinas a partir de um octaedro, as quais afetam diretamente a sua aplicação final (Fuchigami *et al.*, 2020). O íon de Nb^{5+} é também muito grande para caber dentro do octaedro, resultando na formação de estruturas distorcidas tanto nas laterais quanto nos cantos (Ambreen *et al.*, 2021; Taher *et al.*, 2021)

A literatura ainda não é concisa quanto às temperaturas específicas de transição de fases, mas aceita-se que abaixo de 500 °C O Nb_2O_5 tende a formar uma estrutura amorfa, ou seja, sem nenhum arranjo cristalino. Com o aumento da temperatura, o Nb_2O_5 adota uma estrutura pseudohexagonal, chamada de TT- Nb_2O_5 (Figura 4), do alemão Tief-Tief (baixo-baixo), a qual possui mais vacâncias livres para impurezas ocuparem o cristal, como Cl^- e OH^-. A estrutura ortorrômbica Tief (T-Nb2O5) é a segunda cristalização mais conhecida (Figura 5) e, por fim, o H- Nb_2O_5 (Hoch, que significa alto), chamado de monoclínico (Figura 6), quando em temperaturas próximas a 1100 °C (Nico; Monteiro; Graça, 2016a; Nowak; Ziolek, 1999). O H- Nb_2O_5 é o polimorfo mais termodinamicamente estável e é muito pesquisado para capacitores e revestimentos ópticos por ser transparente, enquanto o T-Nb2O5 possui aplicações mais voltadas para a eletroquímica, já que apresenta alta estabilidade química e propriedades elétricas mais acentuadas (Benedito et al., 2024; Nico; Monteiro; Graça, 2016; Zhou et al., 2020)

Essa nomenclatura provinda do alemão foi proposta em 1966 por Schäfer, Gruehn e Schulte (1966), e está relacionada com as condições de síntese. Contudo, até hoje ainda não é considerada padrão, uma vez que se pode encontrar na literatura diferentes classificações, seja pela estrutura cristalina ou pelo formato das partículas, o que torna mais complexa ainda a compreensão do Nb_2O_5 como um todo (Nico; Monteiro; Graça, 2016). De acordo com Skrodczky e colaboradores (2019), há mais de 15 estruturas cristalinas diferentes de Nb_2O_5, incluindo fases metaestáveis. Concomitantemente, de acordo com Ko e Weissmann (1990), as temperaturas de transição de cristalinidade devem ser apenas um indicativo e não um valor absoluto, já que o processo de síntese, temperatura, pH e reagente inicial são alguns dos fatores que determinam o produto, gerando diferentes Nb_2O_5 de acordo com o processo. Assim, se faz necessária uma síntese controlada, especialmente se o Nb_2O_5 for utilizado para algum processo subsequente que requer uma cristalização específica deste material.

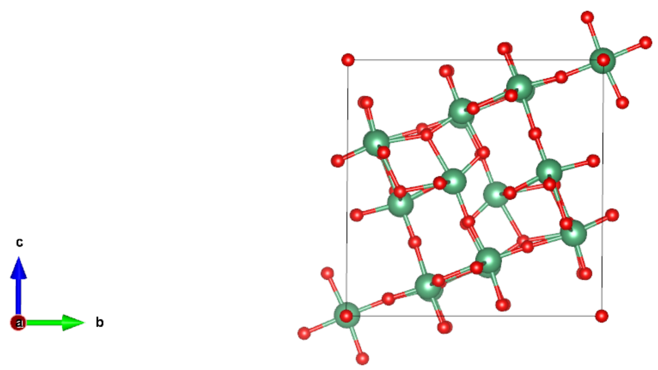

Figura 4: Estrutura cristalina pseudohexagonal (TT-Nb_2O_5) do ON.

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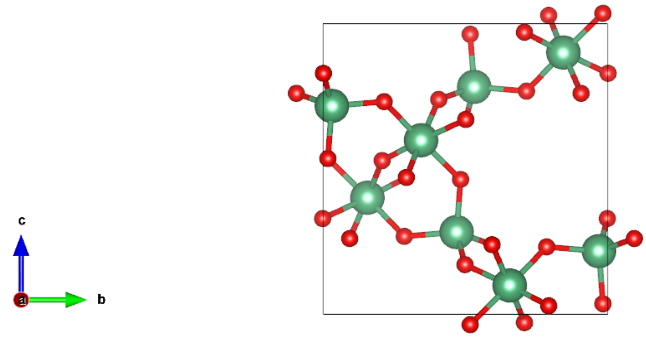


Figura 5: Estrutura cristalina ortorrômbica (T-Nb_2O_5) do ON.

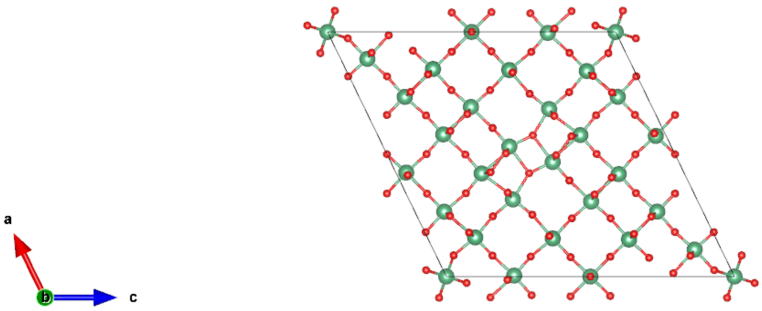


Figura 6: Estrutura cristalina monoclínica (H-Nb_2O_5) do ON.

O Nb_2O_5 apresenta uma alta área superficial, e sua seletividade se dá por consequência da presença de sítios ácidos na sua superfície (De Andrade *et al.*, 2014), podendo ser fortes ou fracos, dependendo da síntese realizada. Diante disso, e somado à sua dificuldade de solubilização em reagentes comumente utilizados, é um candidato interessante na utilização de processos catalíticos em alquilações (Rathnayake *et al.*, 2020), produção de biocombustível (Chhabra; Krishnan, 2023) e desidratação de glicose (Eblagon et al., 2020), por exemplo. A modificação superficial para a adição de novos sítios ácidos também se mostra bastante (Bassan *et al.*, 2013; Eblagon *et al.*, 2023)

As reações redox que ocorrem no Nb_2O_5 normalmente se dão pelos estados Nb^{4+}/Nb^{5+}, proporcionando um comportamento pseudocapacitivo (armazenamento de cargas elétricas eletroquimicamente por reações redox entre um eletrodo e um eletrólito) (Wang *et al.*, 2017) Essas reações ocorrem na superfície do eletrodo (Nb_2O_5), sendo necessário o estudo do comportamento da área superficial do material. Beknalkar e colaboradores (2025) apontam a necessidade de estudar diferentes modificações superficiais no Nb_2O_5 e/ou o desenvolvimento de materiais mistos (como o grafeno e polímeros) que apresentem um comportamento sinérgico.

3.1.4 Importância e aplicações do óxido de nióbio em materiais e biomedicina

A utilização de metais remonta a períodos anteriores à Cristo, sendo encontrados registros em purificação espiritual no Egito e em práticas da medicina indiana Ayurveda destinadas à melhoria da memória. Ademais, existem registros da utilização de ouro (Au), ferro (Fe) e ossos de animais na substituição de partes do corpo (Manivasagam; Dhinasekaran; Rajamanickam, 2010). Até o presente momento, a utilização dessa prática permanece frequente, embora com o devido avanço da tecnologia. A Organização Mundial de Saúde (OMS) divulgou, em 2017, que cerca de 40 milhões de pessoas requerem o uso de próteses ou órteses, sendo que apenas 1 em cada 10 tem acesso a esse serviço.

A aplicação de biomateriais na medicina é de extrema importância, principalmente na área prostética, podendo ser sintéticas, como cerâmicas e metais, ou naturais, como tecidos. Um biomaterial possui a capacidade de interagir com sistemas biológicos e desempenhar o papel de alguma parte do corpo humano, sem alterar significativamente a homeostase, ou seja, causar o mínimo de (ou nenhuma) reação alérgica, imunológica, ou a liberação excessiva de íons. Nenhum deve ser considerado completamente inerte, mas cada material apresenta um determinado nível de inerticidade (Manivasagam; Dhinasekaran; Rajamanickam, 2010; Sowa; Simka, 2018; Sukaryo; Purnama; Hermawan, 2016)

A busca por novos candidatos promissores para a engenharia biomédica é constante, visando tanto a redução de custos como a melhoria do tempo de serviço. O Nb_2O_5 apresenta-se como uma alternativa interessante, visto que é inerte, possui resistência à corrosão e alta força mecânica. Diversos estudos avaliam a utilização do

Nb_2O_5 como revestimento de materiais já utilizados comercialmente (Eduok, 2020; Safavi *et al.*, 2023; Wadullah; Talib Mohammed; Khalid Abdulrazzaq, 2022)). Além disso, o entendimento da atividade que ocorre na superfície de um material e de seu revestimento é de grande importância, visto que é nela que ocorre a primeira interação entre fluidos corporais e o biomaterial.

Na realização de uma pesquisa em sites de busca convencionais e em plataformas acadêmicas, não foi identificado nenhum biomaterial com a presença de Nb ou Nb_2O_5 na sua composição que já esteja comercialmente disponível. Diversos estudos, entretanto, têm como objetivo elucidar a participação destes, de forma que, a longo prazo, seja viável o desenvolvimento de um biomaterial contendo Nb ou ON, como já se encontram estudos para o carregamento mais eficiente de baterias de lítio (Yi *et al.*, 2021), sensores eletroquímicos para a detecção de câncer (Keerthika Devi *et al.*, 2023) desenvolvimento de supercapacitores (Beknalkar *et al.*, 2025), produção de H_2 via catálise (Santos *et al.*, 2024).

Na esfera biomédica, pode-se encontrar uma literatura considerável sobre o revestimento de outros metais com Nb_2O_5, como o aço inoxidável 316 L (Nagarajan; Raman; Rajendran, 2010), ligas de níquel-titânio (NiTi) (Safavi; Khalil-Allafi; Visai, 2023) e magnésio AZ31 (Amaravathy *et al.*, 2014), além da investigação sobre a proliferação celular em contato com o Nb_2O_5 (de Almeida Bino *et al.*, 2021a), genotoxicidade (Schardosim *et al.*, 2022) e efeito *in vivo* (Dsouki et al., 2014). Contudo, são poucos os estudos que avaliam parâmetros biológicos do Nb_2O_5 puro, torna imprescindível uma atenção cuidadosa a essa lacuna.

3.1.5 Relevância da modificação por surfactantes para aplicações específicas

Um dos grandes problemas da utilização de nanopartículas (NP) de óxidos metálicos é a tendência em formar aglomerados em consequência das suas características (Shah *et al.*, 2017), como a alta área superficial e sítios ácidos. Assim, a modificação dessas partículas aparece como uma alternativa para tentar resolver ou melhorar esse problema.

Há duas categorias de alterações na superfície do NP, dependendo do momento em que os surfactantes são adicionados: a primeira é a *in-situ*, que acontece durante o processo de síntese; e a segunda é a modificação pós-síntese, que ocorre após a conclusão da síntese do NP. A maior diferença observada entre as duas tende a ser a formação de partículas com diâmetros menores quando se utiliza a primeira alternativa, uma vez que irão atuar também no impedimento de aglomeração (Iijima; Kamiya, 2009; Shah *et al.*, 2017)

Um surfactante é uma substância que reduz a tensão superficial de uma solução. Tais moléculas são anfifílicas (Figura 7), ou seja, contém regiões hidrofóbicas (geralmente a cauda) e hidrofílicas (uma cabeça polar). As modificações superficiais se baseiam, geralmente, na interação do grupo hidrofílico com a superfície da NP a partir de interações com a superfície. Essas alterações podem não apenas melhorar a aglomeração e consequente estabilidade, mas também possibilitar uma maior compatibilidade das partículas com matrizes orgânicas e organismos biológicos (Heinz et al., 2017; Shaban; Kang; Kim, 2020).

As modificações superficiais com surfactantes podem ser alcançadas tanto por adsorção química quanto física, a depender dos grupos funcionais presentes. A adsorção química ocorre principalmente na presença de grupos tióis (Heinz *et al.*, 2017), estabelecendo ligações covalentes entre as substâncias, com a liberação de um próton. Por outro lado, na adsorção física ocorre a formação de interações eletrostáticas, normalmente com a parte hidrofílica, o que propicia um aumento na afinidade da NP com moléculas orgânicas (Choi *et al.*, 2020)

Surfactantes podem ainda ser classificados de acordo com sua carga: iônicos, que se subdividem em aniônicos, catiônicos e anfotéricos, ou não-iônicos. Os aniônicos apresentam uma carga negativa na porção hidrofílica quando dissociados; os surfactantes catiônicos liberam uma carga positiva, e os anfotéricos conseguem se dissociar em ambos cátions e ânions. Alguns exemplos de grupos iônicos são o sulfato (SO_4^-) presente no dodecil sulfato de sódio (SDS), e a amônia quaternária (R_4N^+) no brometo de cetrimônio (CTAB). Os surfactantes não-iônicos não formam nenhum íon quando em solução aquosa, e são categorizados de acordo com o seu grupo hidrofílico (Ngouangna *et al.*, 2022; Shaban; Kang; Kim, 2020)

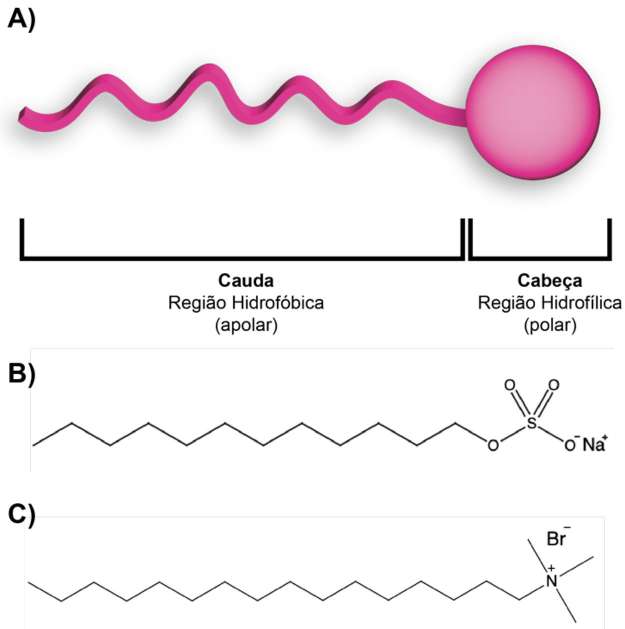


Figura 7: A) Esqueleto geral de um surfactante; B) Estrutura do SDS; C) Estrutura do CTAB.

A utilização de surfactantes para o controle de distribuição de tamanho de NP é fundamental em áreas como a de sensores, uma vez que se precisa identificar traços muito pequenos de material (Shaban; Kang; Kim, 2020) Nanomicelas de quercetina com o surfactante Pluronic-F127 foram avaliadas para a detecção de íons de Cu^{2+}, os quais podem estar associados com condições neurológicas (Jiang *et al.*, 2018). A produção de uma NP sensível a células cancerígenas também foi desenvolvida, utilizando-se da mesma técnica para o controle de tamanho das mesmas (Yang *et al.*, 2021)

O tamanho da partícula de NP de prata (AgNPs), por exemplo, foi influenciado pelo comprimento da cauda hidrofóbica dos surfactantes empregados na sua síntese: à medida que cauda aumentou de 12 para 16 carbonos, o tamanho das NP diminuiu (Abd-Elaal *et al.*, 2018). Yoo e Choi (2010) demonstraram que a síntese de Nb_2O_5 com a adição de SDS, resultou em um aumento no diâmetro de poros das partículas, em parte devido à proteção da superfície causada pela interação eletrostática do surfactante na superfície e pela formação de ligações duplas entre o Nb=O, sendo a concentração mais eficaz de 8.2×10^{-7} M para 520 nm.

A síntese de estruturas em forma de haste (*nanorods*, em inglês) de Nb_2O_5 com a adição de diferentes surfactantes (aniônicos ou catiônicos) evidenciou a influência na morfologia, dispersão e cristalinidade. O cis-1-Amino-9-octadeceno (oleilamina, OAm) é um surfactante catiônico que favoreceu o comprimento dos *nanorods* de ON em 72 ± 14 nm, enquanto o CTAB foi de apenas 46 ± 9 nm. Os surfactantes aniônicos apresentaram valores ainda menores, com SDS em 25 ± 9 nm e o ácido oleico (OA) 36 ± 5 nm, sendo este último, apesar do menor comprimento, o surfactante que apresentou o maior rendimento de *nanorods* (90%), em contraste com a OAm, com 75%, demonstrando a funcionalidade dos grupos específicos de cada surfactante (Ali; Nazemi; Gates, 2017).

Pisárčik e colaboradores (2018) observaram o mesmo comportamento ao utilizar surfactantes catiônicos incluindo o CTAB, brometo de tetradeciltrimetilamônio (TTAB) e outros surfactantes em AgNPs. Os autores também observaram que a adição de surfactantes melhorou a concentração inibitória mínima (CIM) contra bactérias (*Staphylococcus aureus* e *Eschericia coli*) e contra a levedura *Candida albicans*, e que surfactantes contendo um grupo amônia substituído com dibutil ou piridina apresentaram os melhores valores de CIM, possivelmente pela hidrofobicidade e presença de grupos heterocíclicos aromáticos.

AgNPs que foram modificados superficialmente com CTAB, SDS ou Triton X-100 demonstraram também uma redução de 37% na morte celular de *Fagopyrum esculentum* (uma espécie de trigo) quando comparados ao controle sem nenhum surfactante (Oleszczuk; Jośko; Skwarek, 2015). Além disso, houve menos bioacumulação de Ag, sendo a ordem de uso Triton X-100 > CTAB > SDS. O Triton X-100 também foi capaz de reduzir a toxicidade de NP de óxido de titânio (TiO_2) na alga *Daphnia magna*, bem como a adição de CTAB em NP de óxido de zinco (ZnO) (Oleszczuk; Jośko; Skwarek, 2015)

3.2 Caracterização Físico-Química de Materiais Baseados em Óxido de Nióbio

3.2.1 Técnicas de caracterização: FTIR, Raman, TPD, SEM e suas aplicações

A caracterização de um material em fase de pesquisa básica desempenha um papel muito importante no avanço da ciência como um todo, pois várias áreas podem se beneficiar de um mesmo estudo. O Nb_2O_5 tem despertado uma grande atenção não apenas pelo apelo econômico ao injetar o Brasil no cenário mundial, mas por suas propriedades únicas, como comportamento ácido, inerticidade, estabilidade térmica e anticorrosão. A compreensão de seus polimorfos é, portanto, essencial para uma melhor otimização de suas aplicações. Para isso, deve-se empregar diferentes técnicas de caracterização para que se tenha uma visão geral do Nb_2O_5 (Beknalkar *et al.*, 2025; Nico; Monteiro; Graça, 2016a)

Certas metodologias empregadas têm como objetivo analisar as ligações químicas que estão presentes no material, como é o caso da Espectroscopia no Infravermelho por Transformada de Fourier (FTIR) e da Espectroscopia Raman. A técnica de Dessorção de amônia a temperatura programada (TPD-NH_3) possibilita a análise da acidez do Nb_2O_5, isto é, a identificação da existência de sítios ácidos e a avaliação de sua força (Pang *et al.*, 2023). A Microscopia Eletrônica de Varredura (SEM) é outra ferramenta indispensável na caracterização de materiais, fornecendo imagens da morfologia e topografia, além de permitir investigar a distribuição e o tamanho de partículas. A somatória dessas técnicas permite uma análise vasta das propriedades físico-químicas do Nb_2O_5, favorecendo para um melhor uso desse material promissor (21.3: Scanning Electron Microscopy - Chemistry LibreTexts, [*s. d.*]) .

3.2.2 Área superficial

A compreensão da área superficial (AS) de um material, em especial na área biotecnológica e farmacêutica, é de extrema importância, uma vez que a superfície do material representa o primeiro contato com fluidos do organismo humano. Existem diferentes técnicas para avaliar a AS, sendo uma das mais utilizadas a técnica de Brunauer-Emmett-Teller (BET). O BET consiste em uma aprimoração do cálculo de Langmuir, o qual postula que há apenas uma monocamada de adsorção na superfície; assim, os sítios ativos poderiam adsorver apenas uma molécula, sem interação entre elas. A teoria de BET, portanto, assume que há uma adsorção em multicamadas (Figura 8), possibilitando a sobreposição destas, além de fornecer outros parâmetros como dados de porosidade do material (Dollimore; Spooner; Turner, 1976; Tian; Wu, 2018)

Os materiais analisados pelo BET podem ser classificados de acordo com a sua porosidade. Materiais microporosos, por exemplo, apresentam diâmetro menor que 2 nm e são geralmente cristalinos. Por apresentarem alta AS e pequeno tamanho de poro, são amplamente empregados em estudos de catálise e adsorção. As amostras mesoporosas têm diâmetro de poro entre 2 e 50 nm, são normalmente amorfas e utilizadas em áreas que necessitem de uma maior porosidade, como é o caso da liberação de fármacos no *drug delivery* (Alothman, 2012; Eftekhari, 2017; Galarneau *et al.*, 2018). Os materiais macroporosos são aqueles, portanto, com diâmetro de poro acima de 50 nm.

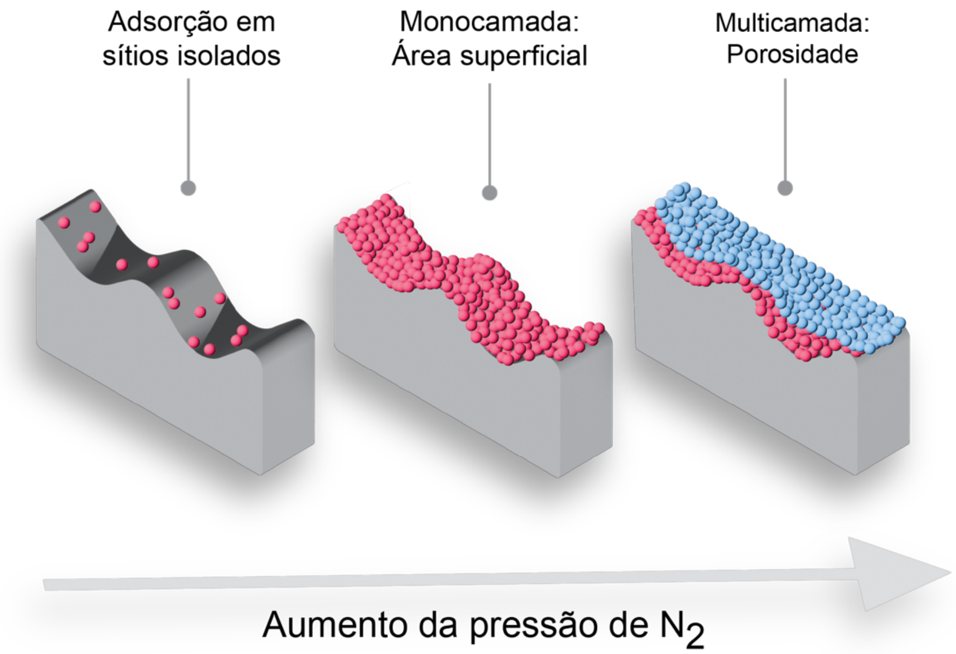
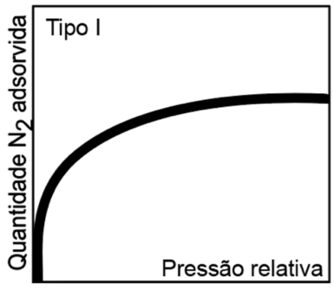
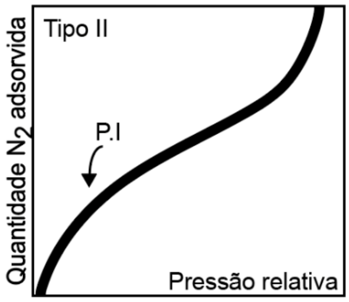
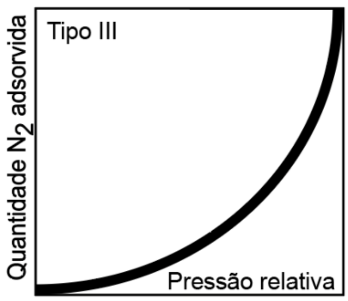


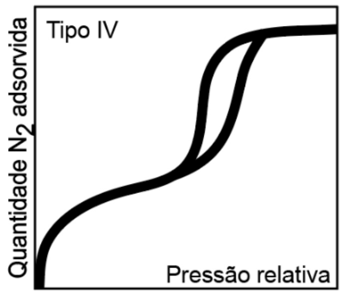
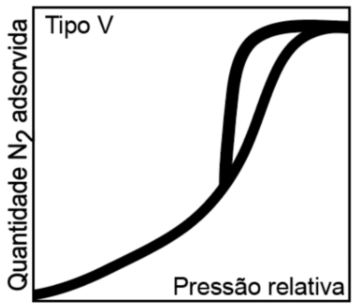
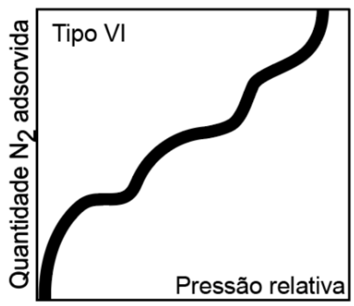
Figura 8: Área superficial e porosidade de um material no BET.

A obtenção da AS se dá por meio de uma isoterma, geralmente utilizando gás nitrogênio N2 em sua temperatura de ebulição (-196 °C). A interação com a superfície do material pode ser tanto física, por meio de Van der Waals, como por resultado de interações químicas. Pode haver seis diferentes classificações de isotermas, divididas em tipos de I a VI, de acordo com a IUPAC, e categorizadas de acordo com características como porosidade e interação adsorvente-adsorvato. A Tabela 2 apresenta as características de cada uma (Ambroz *et al.*, 2018; Thommes *et al.*, 2015).

Tabela 2: Tipos de isotermas

Tipo de Isoterma	Descrição	Formato

Tipo I	Reversível, normalmente encontrada em sólidos microporosos, possui dois subtipos: - Tipo I(a): materiais com largura de microporos < ≈1 nm; - Tipo I(b): para sólidos com microporos mais largos e mesoporos estreitos (< ≈2,5 nm).	
Tipo II	Reversível para materiais não porosos ou macroporosos; - Primeiro ponto de inflexão indica a cobertura da monocamada; - Mudança acentuada na curvatura indica conclusão da cobertura da monocamada; gradual indica sobreposição	
Tipo III	Materiais não porosos e macroporosos; Ocorre quando as interações entre adsorvente e adsorvato são fracas; Não fornece informações sobre a cobertura/formação da monocamada.	

Tipo IV	Dois subtipos: - Tipo IV(a): Largura > tamanho crítico (características de adsorção dependem do material e temperatura). - Tipo IV(b): materiais com mesoporos menores.	
Tipo V	Materiais mesoporosos; Observada em baixas pressões, similar ao Tipo III devido a interações fracas entre adsorvente e adsorvato. - Em pressões mais altas, ocorre histerese (como no Tipo IV(a)); há aglomeração molecular.	
Tipo VI	Materiais não porosos e macroporosos; Comum em adsorção de multicamadas em superfícies não porosas altamente uniformes; - Curva em degraus que depende do material, do gás e da temperatura.	

A AS do Nb_2O_5 foi alterada de 155 m^2/g para 127 m^2/g quando o tempo de calcinação da amostra foi aumentado (Rathnayake *et al.*, 2020), embora ambos tenham exibido a mesma isoterma (tipo IV). Arpini e colaboradores (2019) também observaram a redução da AS do Nb_2O_5 quando e sua temperatura de calcinação foi aumentada de 115 ºC (220 m^2/g) para 300 ºC (114,5 m2/g). A AS do Nb_2O_5 amorfo (450 ºC) foi de 129,6 m^2/g e, com o aumento da temperatura, também se observa a redução da AS: para o Nb_2O_5 a 600 ºC a AS foi de 57,8 m^2/g e para o Nb_2O_5 a 800 ºC, de 43 $m^{2`}$/g (Cao e colaboradores, 2015).

As diferenças no processo de síntese do Nb_2O_5 causam essas alterações na morfologia do material. A síntese por sol-gel tradicional e pelo método de Pechini, e variando também o solvente utilizado, geraram valores significantes: utilizando-se NH_4OH, a AS para o sol-gel foi de 1,70 m^2/g, enquanto para o método de Pechini, foi de 75,85 m^2/g. Para o método de sol-gel, com o aumento da temperatura também se observou a diminuição na AS, mas para Pechini não houve uma sequência lógica (Raba; Bautista; Murillo, 2016)

3.2.3 Sítios ácidos

De acordo com a IUPAC, um sítio ácido de Brönsted é definido como uma molécula capaz de doar um próton (H^+) a uma base ou a uma espécie química (IUPAC, 2008) como o ácido clorídrico (HCl) ou o ácido sulfúrico (H_2SO_4). Um sítio ácido de Lewis é definido como um aceptor de pares de elétrons e capaz de reagir com bases de Lewis, formando um aduto de Lewis ao compartilharem o par de elétrons (IUPAC, 2008) Em outras palavras, a acidez de Brönsted pode ser avaliada a partir da dissociação ácida em água (ou outro meio específico), enquanto a acidez de Lewis se relaciona à constante de associação dos adutos. Entretanto, não existe uma preferência quanto à escolha de um melhor tipo de acidez. A necessidade das reações e procedimentos são os fatores determinantes, visto que reações envolvendo neutralizações ácido-base, por exemplo, priorizam a utilização de ácidos de Brönsted.

A acidez pode ser dividida em categorias de fraca, moderada e forte, em relação à sua força, e em natureza como Brönsted ou Lewis. A força de um material ácido pode ser avaliada pela técnica de dessorção de amônia a temperatura programada (TPD-NH_3), de acordo com os picos nas temperaturas obtidas. Resumidamente, há uma adsorção de NH_3 na superfície da amostra a temperatura constante e, em seguida, uma rampa de temperatura é aplicada para dessorver o gás. Quanto maior a temperatura necessária para retirar o NH_3, mais forte é a acidez do material. Os valores do software fornecem a curva e o valor total da acidez, mas técnicas matemáticas como a deconvolução permitem obter os picos específicos para cada intervalo de temperatura (Bergamaski, [s. d.]; Pang et al., 2023).

Em geral, baixas temperaturas sugerem a presença sítios ácidos mais fracos, como sítios de Lewis, enquanto temperaturas elevadas podem indicar a presença de sítios ácidos de Brönsted, os quais costumam ser mais fortes pelo tipo de ligação que ocorre entre os íons/átomos. Contudo, essa não é uma regra, visto que pode ocorrer dos dois tipos coexistirem em um mesmo pico, necessitando de técnicas mais avançadas para avaliar a natureza de cada sítio, como a espectroscopia de infravermelho da transformada de Fourier de piridina, de acordo com os picos vibracionais específicos que cada um proporciona (Osuga *et al.*, 2017; Precisvalle *et al.*, 2023)

A teoria de Brönsted estabelece que qualquer solvente tem a capacidade de ceder e receber prótons, não se restringindo apenas a soluções aquosas. O que irá variar entre eles é a intensidade do ácido e/ou da base. Na autodissociação da amônia, por exemplo, se demonstra que, similar à autodissociação da água, o íon amônio (NH_4^+) é o ácido mais forte, enquanto NH_2^- é a base mais forte (Figura 9). A água é considerada uma substância anfiprótica, pois pode atuar tanto como base como ácido de Brönsted. A principal característica dessa teoria é o equilíbrio que se estabelece entre a base e o ácido, o qual é alcançado de forma rápida. (Baader, 2011; Eliana Midori Sussuchi; Danilo Oliveira Santos, 2021)

$$2NH_3 \longrightarrow NH_4^+ + NH_2^-$$

$$2H_2O \longrightarrow OH^- + H_3O^+$$

Figura 9: Autodissociação da amônia e da água.

A teoria de Lewis se mostra muito mais abrangente do que a de Brönsted, pois engloba uma gama maior de compostos, uma vez que, em vez de se basear na doação de um próton, caracteriza-se pelo compartilhamento de pares de elétrons, resultando na formação de um complexo, também chamado de aduto. Um ácido de Lewis será o composto que irá aceitar o compartilhamento, e a base de Lewis será a doadora. Dessa forma, pode-se associar que, ao unir as duas teorias, o próton de Brönsted seria o aceptor do par de elétrons da base de Lewis. Esta explicação pode ser demonstrada pela protonação da amônia, como demonstra a Figura 10(Baader, 2011; Eliana Midori Sussuchi; Danilo Oliveira Santos, 2021).

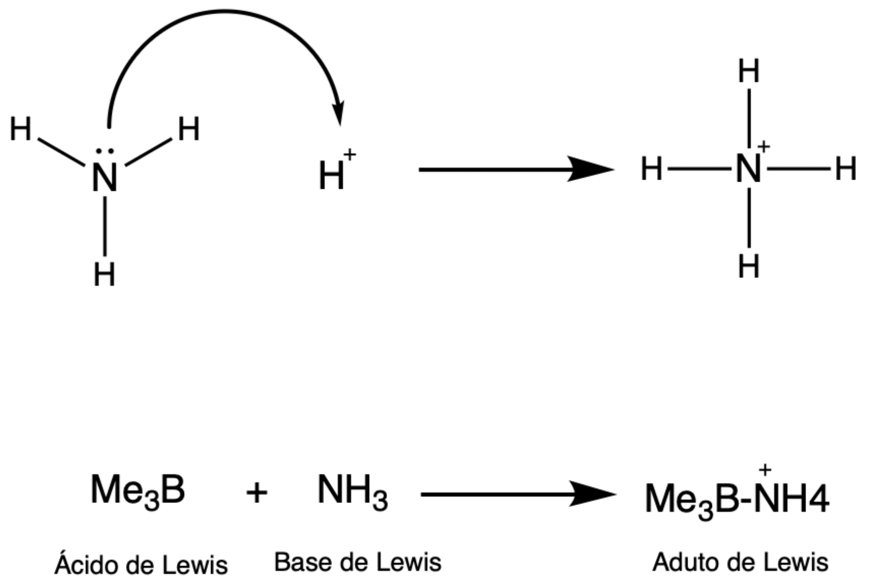


Figura 10: Protonação da amônia e formação do aduto de Lewis.

A acidez de um material metálico pode ser determinada pela sua disposição na tabela periódica, considerando três parâmetros: (1) quanto maior a carga do metal, mais ácido ele vai ser; por conseguinte, o Al^{3+} é mais ácido que o K^{+}; (2) ao analisar o raio atômico, observa-se que quanto menor este raio, mais ácida será a substância; portanto, ao se descer na tabela periódica, os metais são menos ácidos; (3) no que se refere aos metais de transição, quanto mais eletronegativo for, mais forte será o ácido. A utilização desta propriedade em sistemas biológicos, ainda que não tenham sido encontrados estudos relacionando sítios ácidos, se faz necessária, uma vez que moléculas e micro-organismos também possuem uma carga. Mudanças nas membranas celulares podem alterar a sinalização celular, como também alterações nas cargas dos aminoácidos, que levarão a uma proteína defeituosa (Baader, 2011; Goldenberg; Steinberg, 2010; LibreTexts Chemistry, [*s. d.*])

Nas pesquisas de *drug discovery*, sabe-se que compostos não ionizados tendem a atravessar a membrana lipídica mais facilmente por serem moléculas mais lipofílicas. Moléculas ácidas apresentam maior biodisponibilidade oral, e bases tendem a se protonar no trato gastrointestinal, diminuindo a sua biodisponibilidade por alterar a polaridade e a lipofilicidade. Desta forma, a carga de uma molécula influencia em como esta interage com os alvos no organismo, bem como seu metabolismo, excreção e toxicidade (Gleeson, 2008; Manallack *et al.*, [*s. d.*])

Uma investigação na área de cristalografia 3D investigou a estrutura da chimotripsina, uma enzima envolvida na digestão, e verificou que determinados aminoácidos ficavam mais próximos entre si na estrutura tridimensional do que em sua sequência primária, como ocorre com a histidina 57 e a serina 195. Essa proximidade resulta em uma ligação de hidrogênio entre o grupo imidazol e hidroxila, respectivamente, onde a histidina age como uma base de Brönsted ao receber um próton do grupo OH^-, tornando a serina, que agiu como ácido de Brönsted, mais reativa, facilitando uma melhor catálise das ligações peptídicas (Blow; Birktoft; Hartley, 1969; James J Neitzel, 2010).

A anidrase carbônica humana II é uma enzima que atua no transporte de gás carbônico (CO_2) e no controle de pH do sangue. Essa enzima necessita do Zn para seu funcionamento no sítio ativo, o qual é coordenado à proteína por três grupos imidazois de resíduos de histidina e uma molécula do solvente (a depender do pH). Esse íon de Zn atua, na proteína, como sítio de Lewis, de modo a baixar o *pK*a da ligação Zn-H_2O e formar um complexo Zn-OH^-, transferindo também um próton para o resíduo de histidina, garantindo a reatividade do sítio catalítico. Dessa forma, consegue realizar a conversão de CO_2 em HCO_3^-, mantendo a homeostase sanguínea (Carlsson; Jonsson, 2000; Kyun Kim *et al.*, 2018)

3.2.4 Nb_2O_5 como material ácido

Compostos que possuem geometria de oxidação 5+ tendem a ser ácidos fortes de Lewis, formando um octaedro ou um ânion quando ocorre o compartilhamento de elétrons. O Nb_2O_5, por exemplo, é um material que possui alta carga de oxidação (5+) e baixo raio iônico (O.68 Å), constituindo um alvo interessante para estudos de acidez. Ademais, o ON é termodinamicamente estável e pode ser modificado superficialmente conforme a síntese utilizada. Tanabe (2003) demonstrou que, ao ser aquecido a baixas temperaturas e hidratado ($Nb_2O_5.nH_2O$, até 300 °C), apresenta acidez comparável a 70% da atividade do ácido sulfúrico H_2SO_4.

Ouqour e colaboradores, nos anos 90, estudaram pioneiramente o comportamento do ON como catalisador através da conversão de propan-2-ol em propano e acetona.

Os autores observaram que o ON pode agir tanto como ácido de Brönsted quanto de Lewis, dependendo da temperatura de calcinação à qual foi exposto (99.5, 199.85, 299.85, 399.85, 499.85 °C), apresentando maior número de sítios ácidos (28.1×10^{-7} mol/m^2) a 399.85 °C e decaindo significativamente a 499.85 °C (11.4×10^{-7} mol/m^2). Esse comportamento foi relacionado pelos pesquisadores com o maior número de sítios de Brönsted para uma conversão mais efetiva do propano-2-ol, possivelmente pela necessidade de remover água do álcool, facilitando a reação pela doação de prótons, enquanto sítios de Lewis poderiam causar reações secundárias (Nash *et al.*, 2015).

O FTIR de piridina indicou a presença de ambos os sítios ácidos no ON a 00 °C e, conforme o aumento da temperatura de calcinação, menor a quantidade de sítios e a força de Brönsted (Bassan *et al.*, 2013). A presença de água ou de outro solvente pode resultar na formação desses íons, os quais perdem o sinal na análise à medida que a temperatura de calcinação se eleva (Iizuka, Ogasawara, Tanabe, 1983). Hanaoka e colaboradores concordam com estes resultados e determinam que os sítios de Lewis estão relacionados com a reação de isomerização e abertura de anel aromático, uma vez que as temperaturas coincidem.

3.2.5 Espectroscopia Raman

A espectroscopia de Raman é uma técnica utilizada para analisar mudanças vibracionais e rotacionais em uma amostra, fundamentando-se no espalhamento de uma luz monocromática, tipicamente um laser. A energia produzida pelas vibrações moleculares ou outros tipos de excitação são capazes de fornecer a identidade molecular do composto em questão. É uma técnica empregada em diferentes áreas do conhecimento devido à sua propriedade não-destrutiva(Kaewseekhao *et al.*, 2020; Orlando *et al.*, 2021). A maior parte da luz dispersa possui o mesmo comprimento de onda da luz incidente no material (denominado de espalhamento Rayleigh), mas uma pequena porção é desviada, a qual é conhecida por espalhamento Raman (Figura 11).

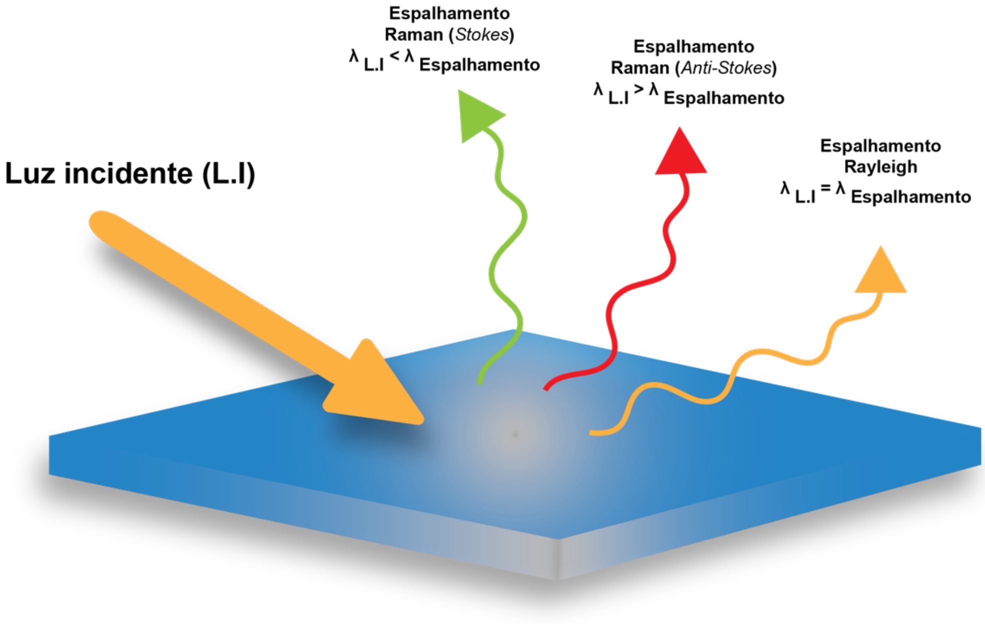


Figura 11: Representação do espalhamento da luz incidente.

A Raman consegue detectar e diferenciar, por exemplo, a forma ativa e latente da tuberculose (Kaewseekhao *et al.*, 2020) com uma acurácia de mais de 90%. Além disso, é capaz de identificar e diferenciar biomoléculas como DNA, proteínas e carboidratos, gerando uma ampla gama de impressões digitais (Kuhar *et al.*, 2018), as quais podem ser comparadas com as mesmas biomoléculas dentro de um contexto envolvendo uma alguma alteração bioquímica. Dessa forma, é possível identificar variações de vibrações que podem indicar causa ou consequência de uma patologia, por exemplo.

As simetrias do Nb_2O_5 também podem ser avaliadas por Raman, e possuem bandas específicas de acordo com cada estrutura. O octaedro NbO_6, mais envolvido nas estruturas cristalinas do Nb_2O_5, possui uma banda entre 850-1000 cm-^{1}, podendo encontrar NbO_7 e NbO_8, que são detectáveis entre 500-700 cm^{-1}, e NbO_4, a 790-830 cm^{-1} (Jehng; Wachs, 1990; Nowak; Ziolek, 1999). Pradhan e colaboradores (2016) avaliaram o espectro de Raman de ON em diferentes temperaturas de calcinação e observaram que, a depender da cristalização, os picos são mais ou menos definidos, mas apresentam uma estrutura similar (Figura 12).

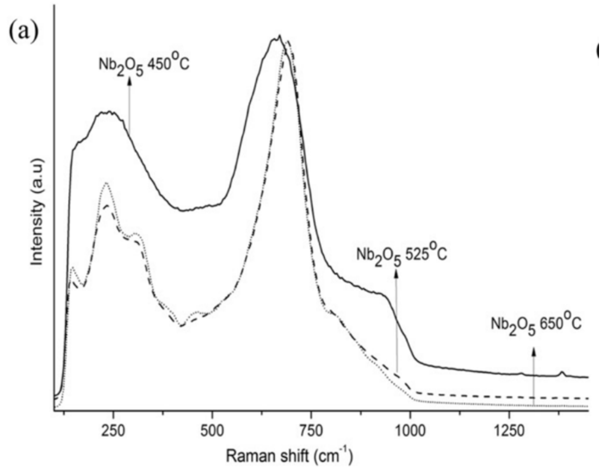


Figura 12: Espectro Raman de Nb_2O_5 calcinados em diferentes temperaturas.
Fonte: Pradhan *et al*. (2016).

Os picos encontrados para a amostra Nb_2O_5-450 °C (ON calcinado a 450 °C) demonstram modelos vibracionais relacionados às ligações Nb-O, e o espectro menos definido é uma característica de uma amostra amorfa. O ON calcinado a 525 °C e a 650 °C apresentam cristalinidades semelhantes, uma vez que os picos se sobrepõem quase completamente, sendo característicos de TT-Nb_2O_5. Observa-se também um pequeno pico próximo a 500 cm^{-1} para o Nb_2O_5-650 °C, e um leve deslocamento no pico próximo a 700 cm-^{1}, associados a uma leve ordenação da estrutura cristalina. Outro estudo também avaliou diferentes temperaturas de calcinação para o ON e observou um comportamento semelhante, ou seja, uma maior definição dos picos no espectro com o aumento da cristalização do material (Ücker *et al.*, 2022)

Ali, Nazemi e Gates (2017) apresentam os espectros de Raman dos *nanorods* de ON com variações vibracionais próximas a 1600 cm^{-1}, resultantes das modificações causadas pela adição de diferentes surfactantes, como C=O e N-H.

3.2.6 Espectrometria por infravermelho com transformada de Fourier

A espectroscopia por infravermelho com transformada de Fourier (FTIR) é uma técnica óptica empregada para avaliar as alterações moleculares resultantes dos movimentos de uma partícula. As moléculas não são estáticas, pois possuem movimento devido às suas ligações, podendo ser simétricos ou assimétricos, e situados dentro ou fora do plano bidimensional (Figura 13). Diferentemente do Raman, que avalia o espalhamento de uma luz monocromática, o FTIR se baseia na detecção vibracional das moléculas, que são específicas para cada ligação. As vantagens do FTIR incluem sua natureza não destrutiva, permitindo a reutilização da amostra após a análise, a ausência de aditivos na análise, sendo também ambientalmente correta, facilidade de operação e alta sensibilidade e acurácia. Entretanto, necessita de uma referência padrão para comparação (Movasaghi *et al*., 2008; Song *et al.,* 2020).

A identificação de grupos funcionais presentes em uma amostra é uma das grandes aplicações do FTIR. A identificação do ibuprofeno em um complexo de NP foi efetiva a partir dos grupos funcionais presentes neste medicamento (Fahelelbom *et al.*, 2023). A junção de glicina com o fio de sutura poligactina (PLGA) foi também corroborada utilizando FTIR para a formação de micelas poliméricas (Madhwi *et al.*, 2017) para a inserção de Metrotexato, um fármaco utilizado para o tratamento de alguns tipos de câncer.

A verificação da autenticidade de drogas é outra aplicação do FTIR na área farmacológica. A identificação de Viagra® e seus genéricos (Custers *et al.*, 2015), bem como antibióticos de países africanos (Alotaibi et al., 2018), e até medicamentos da medicina tradicional chinesa (Kahmann *et al.*, 2018) são alguns dos exemplos. Identificar polimorfismos de medicamentos como a carbamazepina também é de grande importância, de forma a se ter a forma ativa adequada (Krstić *et al.*, 2015).

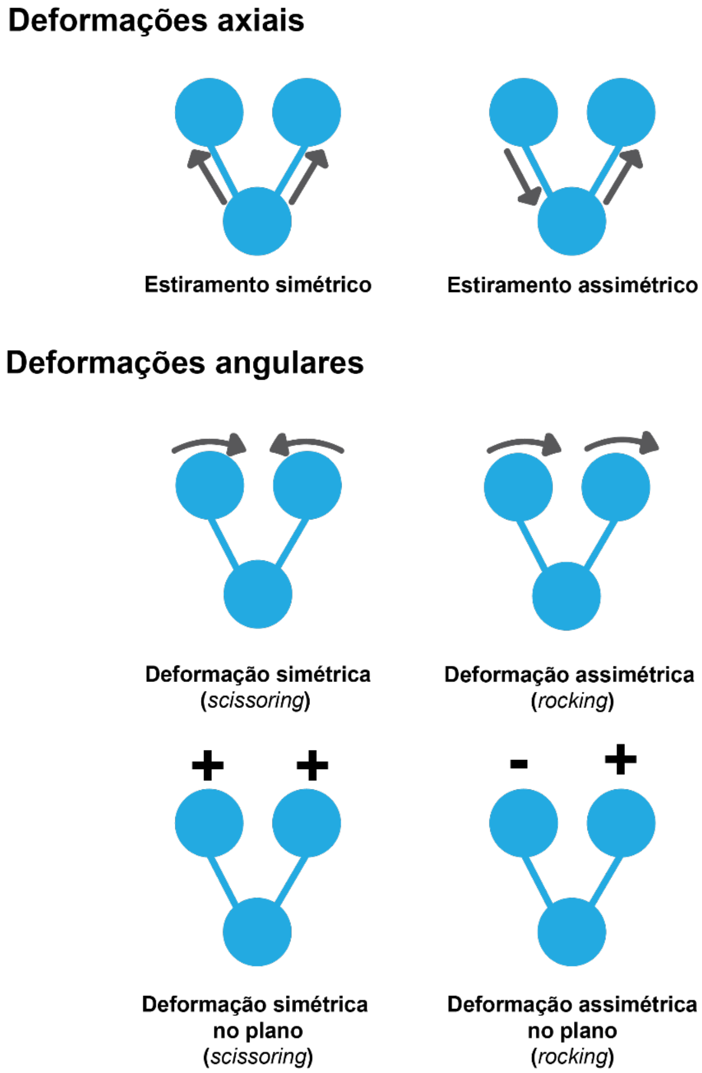


Figura 13: Estiramentos axiais e angulares de moléculas. Imagem gerada no Adobe Illustrator 28.6.

A identificação do Nb_2O_5 no FTIR ocorre a partir de picos específicos no espectro, sendo Nb–O–Nb (800 cm^{-1}), Nb3O (470 cm^{-1}) e Nb–O–Nb (650 cm^{-1}) (Pawlicka; Atik; Aegerter, 1997). A incorporação de grupos hidroxila pode gerar um aumento na espessura dos picos (Nagarajan; Raman; Rajendran, 2010). A presença de água pode ser observada entre 3000-3800 cm^{-1}, indicando que a amostra não foi seca o suficiente. Boukriba e Sediri (2014) ainda atribuem o pico em 814 cm^{-1} para Nb=O. Ucker e colaboradores (2019) também identificaram a presença de água no Nb_2O_5, como uma banda em 1363 cm^{-1} relacionada à adsorção de O^{2-} na superfície em sítios livres, favorecida pelo aquecimento da amostra. A adsorção de grupos NH_4 podem ser visualizados a 1433 cm^{-1} e do grupo peroxo (–O–O–H) próximo a 900 cm^{-1} (Millan; Susrisweta; Sahoo, 2023).

3.2.7 Microscopia Eletrônica de Varredura

Existem dois tipos de microscópio: o óptico (OM), que se utiliza da luz visível como fonte, e o eletrônico (EM), que emprega o uso de um feixe de elétrons acelerado. A superfície de um material pode ser estudada por ambos, porém a resolução do EM favorece o seu uso para esse fim. Ambos possuem a mesma teoria e funcionamento: magnificar objetos que são invisíveis a olho nu, apresentando os seus componentes e estrutura. Diferentemente, o OM consegue operar a pressão atmosférica, enquanto o EM necessita de um sistema de vácuo. A EM divide-se em Microscopia Eletrônica de Varredura (SEM) e Microscopia Eletrônica de Transmissão (TEM): a primeira se caracteriza pelo estudo da morfologia e topografia superficiais de uma amostra, enquanto a segunda permite avaliar a composição interna. Para este projeto de tese de doutorado, o foco será especificamente no SEM o qual foi introduzido teoricamente em 1938 por Von Ardenne, e dentro de 30 anos já se produziu o primeiro modelo (21.3: Scanning Electron Microscopy - Chemistry LibreTexts, [s. d.]; Akhtar et al., 2018).

As texturas, tamanhos, formatos e arranjos do ON podem também ser estudados utilizando as imagens obtidas por SEM. (Li e colaboradores (2016) observaram que o Nb_2O_5 amorfo (a-Nb_2O_5) apresentou menor tamanho de partícula e melhor dispersão (Figura 14), e que a temperatura não só alterou a cristalinidade como o tamanho. Já a adição de peróxido de hidrogênio (H_2O_2) durante a síntese permitiu uma melhor dispersibilidade das partículas. O Nb_2O_5 comercial também foi analisado, sendo possível visualizar diferentes formatos de partículas. O tipo de síntese, como já foi mencionado anteriormente, também influencia na morfologia final. O Nb_2O_5 formado pela técnica de sol-gel tradicional gerou partículas esféricas bem definidas; já o método de Pechini permitiu uma granulação maior na superfície (Raba; Bautista; Murillo, 2016)

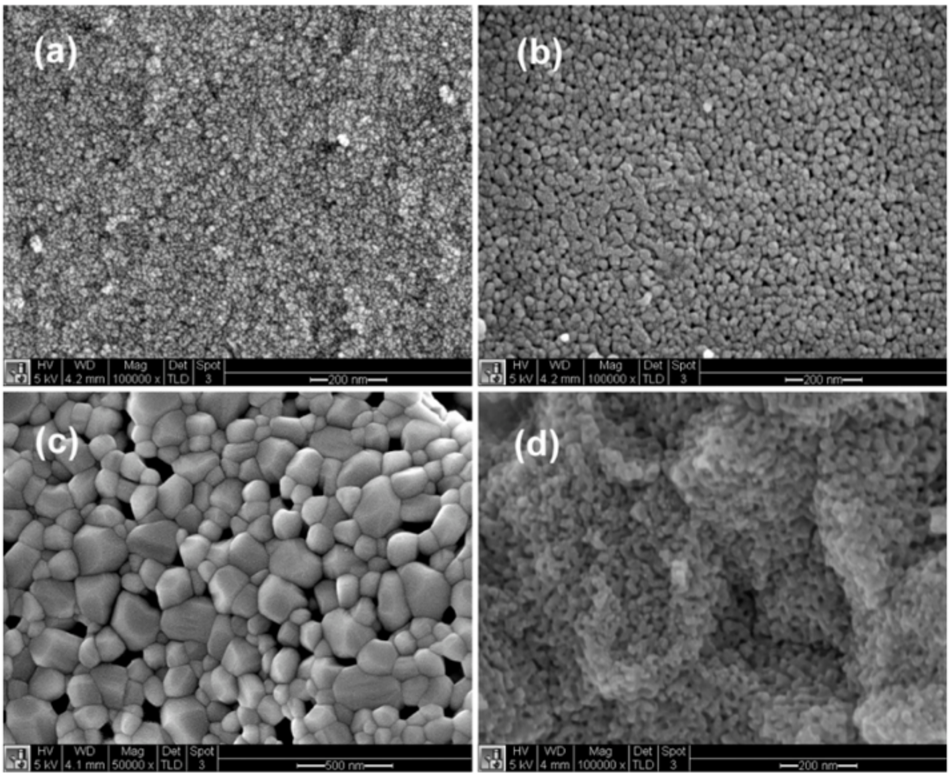


Figura 14: Microscopia Eletrônica de Varredura de diferentes tipos de ON a) comparação de ON amorfo, b) sintetizado com H_2O_2 a 600 ºC, c) sintetizado com H_2O_2 a 800 ºC, d) sem a adição de H_2O_2 a 600 ºC. Fonte: Li e colaboradores (2016).

3.2.8 Cristalinidade

A Difração de Raios X (DRX) é uma técnica analítica não destrutiva frequentemente empregada para analisar as características estruturais de materiais cristalinos. Os raios X, que são feixes de luz de alta energia e comprimento de onda extremamente curto, interagem com os átomos organizados do material em redes cristalinas, sendo a base teórica para essa técnica. Ao atingir um material cristalino, um feixe de raios X é disperso pelos átomos do cristal em ângulos determinados, gerando um padrão de difração distinto. Este padrão, formado por picos de intensidade em variados ângulos, espelha a estrutura atômica e pode ser aplicado para identificar a composição cristalina, estabelecer as características da célula unitária, identificar fases no material e até mesmo calcular o tamanho dos cristais ou o nível de cristalinidade (X-Ray Diffraction for Materials Research: From Fundamentals to Applications Myeongkyu Lee, 2017; X-ray diffraction (XRD) basics and application - Chemistry LibreTexts, [s. d.]).

Os ângulos são chamados de 2θ e representam a diferença angular entre o feixe de raios X incidente e o feixe de raios X difratado. Quanto maior o sinal do pico, maior a quantidade de cristais e, quanto mais estreito o pico, maior o cristal. Portanto, um pico não muito definido pode ser resultado de um cristal defeituoso ou de um material amorfo. Assim como nas técnicas de FTIR e RAMAN, cada cristal apresenta um padrão de difração único, e as fases podem ser comparadas com dados padrões da *Joint Committee on Powder Diffraction Standards* (JCPDS), fundada em 1941. A JCPDS tem como propósito coletar, editar, publicar e distribuir referências padrões para a identificação de materiais cristalinos (The International Centre for Diffraction Data - ICDD, [s. d.]; X-ray diffraction (XRD) basics and application - Chemistry LibreTexts, [s. d.]).

A técnica de DRX é amplamente utilizada, pois permite a identificação e diferenciação entre diferentes fases cristalinas e o grau de desordem de um material, mesmo em amostras complexas ou multicomponente. O Nb_2O_5, por possuir um grande polimorfismo, constitui um exemplo ideal para se utilizar essa técnica, visto que cada cristalinidade possui uma aplicação mais específica, conforme discutido anteriormente (21.3: Scanning Electron Microscopy - Chemistry LibreTexts, [s. d.]; Nowak; Ziolek, 1999).

(Falk e colaboradores 2017) avaliaram três diferentes temperaturas de calcinação do Nb_2O_5: 500 °C, 600 °C e 800 °C. A temperatura inicial evidenciou uma cristalização TT- Nb_2O_5 (JCPDS Nº. 00-007-0061), sendo uma estrutura mista de pseudohexagonal e amorfa; a segunda e terceira temperaturas resultaram na forma ortorrômbica T- Nb_2O_5 (JCPDS Nº. 00-030-0873), enquanto a 800 °C também revelou uma estrutura monoclínica. É possível observar na Figura 15 o aumento da definição dos picos com o aumento da temperatura de calcinação.

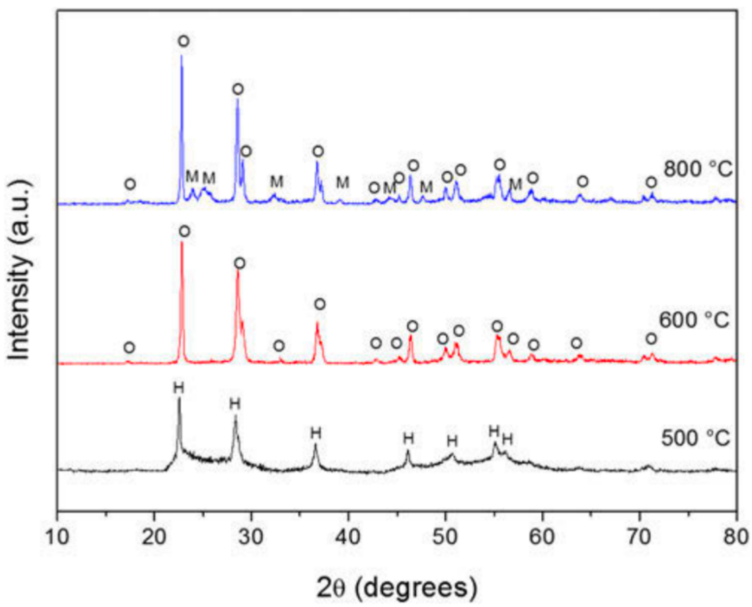


Figura 15: Espectro de DRX para Nb_2O_5 calcinados a diferentes temperaturas.
Fonte: Falk et al., (2017).

3.3 Avalição da atividade *in vitro*

3.3.1 Conceitos básicos de citotoxicidade

O termo “citotoxicidade” descreve a habilidade de um composto/molécula em ser prejudicial a uma linhagem celular, causando danos ou até mesmo a morte. A citotoxicidade pode ocorrer por diversos mecanismos: a indução de alterações na membrana celular, comprometimento de organelas, o bloqueio ou superestimulação de processos metabólicos e ativação de vias apoptóticas. Existem três grupos principais para avaliar a citotoxicidade: colorimétricos, que avaliam a indução da citotoxicidade pela mudança de cor; fluorimétricos, que medem o nível de fluorescência emitido; e exclusão por corante, que avalia uma suspensão celular com um corante, o qual não é capaz de permear a membrana de células viáveis. Os dois primeiros métodos são diretamente proporcionais, ou seja, dependem da viabilidade celular para emitir a coloração ou fluorescência (Bácskay *et al.*, 2017; Chaudhry *et al.*, 2024)

A avaliação da citotoxicidade é comumente realizada na pesquisa básica com o objetivo de determinar os efeitos de compostos químicos, materiais ou nanopartículas em diferentes tipos de células. Métodos como o ensaio de viabilidade celular, como o ensaio colorimétrico do sal (3-(4,5-dimetiltiazol-2yl)-2,5-di-fenil brometo de tetrazolina) conhecido por MTT, o qual avalia a atividade metabólica pela redução deste composto em formazan (Figura 16), um composto que é insolúvel em água e gera cristais de coloração roxa, que são medidos espectrofotometricamente a 570 nm (Mosmann, 1983) Essa reação é mediada por enzimas da cadeia respiratória, como oxirredutase e desidrogenase, presentes nas mitocôndrias das células viáveis (Berridge; Herst; Tan, 2005). Ainda que esse ensaio se atribua à atividade mitocondrial, alguns autores encontraram o formazan em outros locais celulares, como *droplets* lipídicos (Stockert *et al.*, 2012), membrana plasmática (Bernas; Dobrucki, 2000) e até no núcleo (Liu 1997).

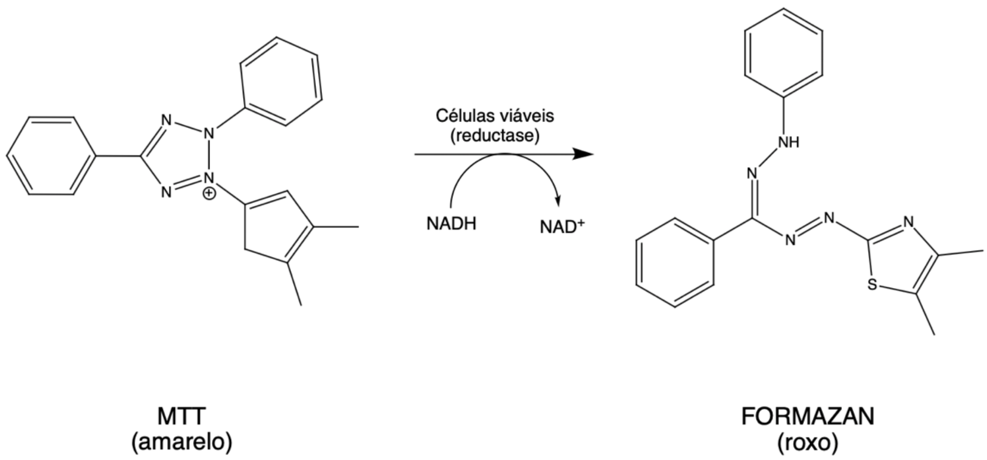


Figura 16: Conversão de MTT em formazan por células viáveis

A atividade hemolítica, ou seja, a avaliação da citotoxicidade em eritrócitos, é um dos protocolos mais simples, embora não menos importante, para avaliar inicialmente a citotoxicidade. Trata-se de um modelo simples pela ausência de núcleo, e por estarem em grande quantidade no organismo. Ademais, a liberação de hemoglobina (Figura 17) é facilmente detectada por espectrofotômetro (Manaargadoo-Catin *et al.*, 2016)). A estrutura do eritrócito é composta aproximadamente por 52% de proteínas, 40% de lipídios e 8% de carboidratos (Dodge; Mitchell; Hanahan, 1963). As proteínas presentes na membrana das hemácias são responsáveis não só pelo transporte de íons, mas pela sua integridade mecânica e estrutural (Manaargadoo-Catin et al., 2016).

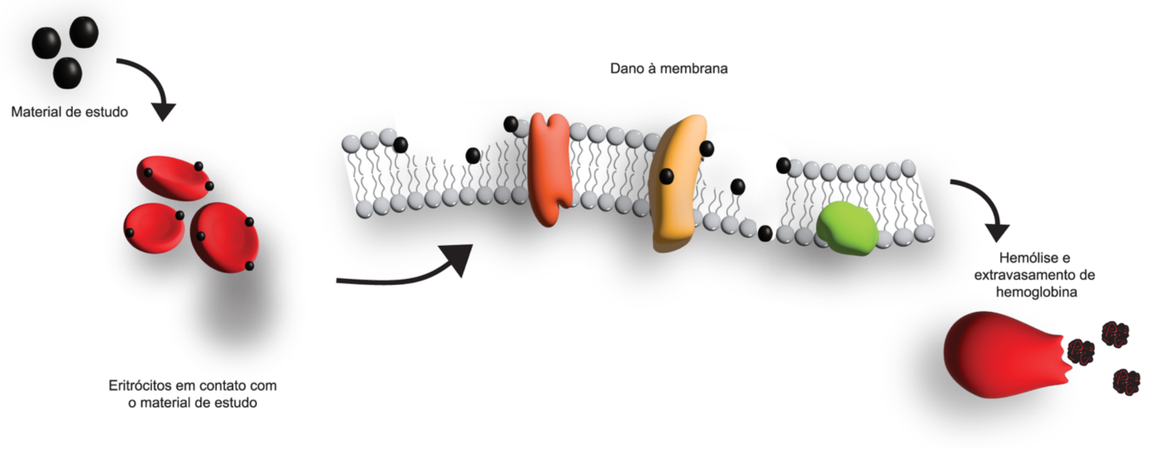


Figura 17: Processo esquemático do rompimento da membrana de eritrócitos.

Na pesquisa farmacêutica, a avaliação da citotoxicidade é fundamental para o desenvolvimento de medicamentos seguros durante o processo de triagem de novos compostos, seja na medicina humana, animal ou na área agrícola. Um passo essencial é a seleção apropriada da linhagem celular de acordo com o propósito do estudo. Atualmente, há diversos bancos de células ao redor do mundo que garantem as linhagens celulares com alta reprodutibilidade, sejam elas imortalizadas ou primárias. Somado a isso, na pesquisa oncológica, a citotoxicidade pode ser estudada de maneira contrária, buscando agentes que causem a morte de células tumorais, enquanto preservam as saudáveis. Dessa forma, o estudo da citotoxicidade não apenas garante a segurança dos medicamentos, mas também contribui para o desenvolvimento de terapias específicas para diferentes doenças (Bácskay *et al.*, 2017; Chaudhry *et al.*, 2024)

3.3.2 Estudos prévios de citotoxicidade do ON

O Nb_2O_5 é um material largamente utilizados em diversos setores, como já discutido em tópicos anteriores. Por se tratar de um material insolúvel, inerte e de grande resistência à corrosão, o estudo da utilização do Nb_2O_5 em biomateriais vem crescendo, em especial no revestimento de próteses metálicas, que serão discutidos neste tópico. Contudo, são poucos os estudos que investigam a atividade desse material em sua forma pura.

A avaliação do Nb_2O_5 em diferentes calcinações (450 ºC, 525 ºC e 650 ºC) foi realizada em uma linhagem de fibroblasto de camundongo (L929), observando-se nenhuma, 64% e 70% de perda na viabilidade no primeiro dia, respectivo às temperaturas (Pradhan; Wren; Mellott, 2017). Já a atividade antitumoral do Nb_2O_5 foi testada em uma linhagem de camundongos geneticamente modificada (L20B) e em linhagem de câncer de mama humano (MCF7), demonstrando um aumento na inibição de 60% e 33.9% quando as células foram expostas a 50 µg/ml, respectivamente (Salim *et al.*, 2022). Schardosim e colaboradores (2022) investigaram o efeito genotóxico de NP amorfas (450 ºC) e cristalinas (550 ºC) em células de câncer de ovário (CHO-K1), observando danos do DNA nas concentrações de 53, 105, e 210 g/mL para as NP cristalinas e, para a amostra amorfa, a 210 g/mL.

O revestimento de materiais metálicos, como na liga AZ31, demonstrou que o ON foi capaz de aumentar a quantidade de células de osteossarcoma humano (MG-6) aderidas de 3200 para 7000 no material (Amaravathy *et al.*, 2014). O aço inoxidável 316 L demonstrou uma proliferação quase duas vezes maior em cementoblastomas (células derivadas do tecido conectivo fibroso do ligamento periodontal) quando revestido com o ON (Ramírez et al., 2011). A liga de titânio Ti6Al4V também apresentou alta viabilidade celular (97%) na presença do Nb_2O_5, em linhagem celular de tecidos renais de macacos-verdes-africanos (Chlorocebus sp.), conhecidas por VERO-CCL81 (de Almeida Bino et al., 2021), além de uma diminuição de fatores apoptóticos de linfócitos, células T *helper* e citolíticas.

Vidros de aplicação biológica, como o 45S5 Bioglass®, também foram analisados na presença de Nb_2O_5 (800 ºC) em células de osteosarcoma (Saos-2), demonstrando um melhor metabolismo com o aumento da porcentagem de Nb_2O_5 (Hammami et al., 2023). Nesta mesma linhagem celular, um material consistindo Nb_2O_5, gelatina e o polímero policaprolactona (PCL) mostrou também um aumento na viabilidade celular e maior metabolismo ao se utilizar 7% wt ON (Marins *et al.*, 2019). Entretanto, o vidro invertido de fosfato modificado com Nb_2O_5 (1400 ºC) não obteve aumento na proliferação celular (Obata et al., 2012), nem o titânio comercial (Li, Yuncang *et al.*, 2016). O material de dimetil polissiloxano (PDMS) com 40% wt Nb_2O_5 apresentou 5 vezes maior viabilidade celular quando comparado ao controle após 3 dias (Young *et al.*, 2014) .

Outros estudos na literatura com diferentes revestimentos ou inserção do Nb_2O_5 em sua composição estão disponíveis na literatura (Queiroz *et al.*, 2021; Safavi;

Khalil-Allafi; Visai, 2023; Samudrala *et al.*, 2018). De forma geral, o Nb_2O_5 apresenta-se como um material promissor, a depender da cristalização e síntese a serem utilizadas. O desempenho da citotoxicidade do Nb_2O_5 em diferentes materiais, metálicos ou não, demonstra a possibilidade de ser um forte candidato para estudos pré-clínicos de biomateriais. Ademais, é imprescindível também esclarecer os mecanismos ainda não compreendidos sobre a interação do Nb e do Nb_2O_5 no organismo.

3.4 Testagem *In Vivo*

3.4.1 Modelos animais alternativos

A utilização de modelos animais mamíferos permanece prevalente na pesquisa científica, devido à sua semelhança fisiológica e anatômica com os seres humanos. No entanto, além de serem modelos caros e laboriosos, suscitam um questionamento ético e social devido ao sofrimento do animal, à privação de liberdade e à utilização de métodos invasivos. Assim, o princípio dos 3R (do *inglês reduction, replacement and refinement*) promove uma adoção de métodos alternativos aos mamíferos (Freires et al., 2017; Mukherjee et al., [s. d.]).

Os modelos animais alternativos vêm ganhando espaço nas pesquisas por serem evolutivamente menos desenvolvidos, resultando em uma nocicepção menos complexa desses animais também. Uma das alternativas é o inseto *Drosophila melanogaster*, conhecido como mosca-da-fruta, e amplamente utilizado na área genética, sendo um dos primeiros animais a ter o seu genoma completo sequenciado. Apresenta vantagens como seu pequeno tamanho, um ciclo de vida curto e alta taxa de reprodução. Além disso, possui cerca de 60% de similaridade com o código genético humano, incluindo mutações e deleções, mas com uma maior simplicidade por conter apenas quatro cromossomos homólogos (Baenas; Wagner, 2022; Severino *et al.*, 2023) .

O tempo entre a fertilização e o aparecimento de larvas leva cerca de um dia, e o indivíduo pode ser considerado adulto com 10 dias. As fêmeas já têm a capacidade de se reproduzir após as primeiras 5-8h, enquanto os machos necessitam de mais de 8h para a maturação sexual. A expectativa média de vida da *D. melanogaster* é em torno de 60 dias. Além disso, apresentam dimorfismo sexual: as fêmeas geralmente são maiores e apresentam listras no abdômen; e o macho é menor e a extremidade do seu abdômen é preta (Figura 18). Outra diferença consiste no “pente sexual” localizado no primeiro par de patas do macho(Baenas; Wagner, 2022; Pandey; Nichols, 2011).

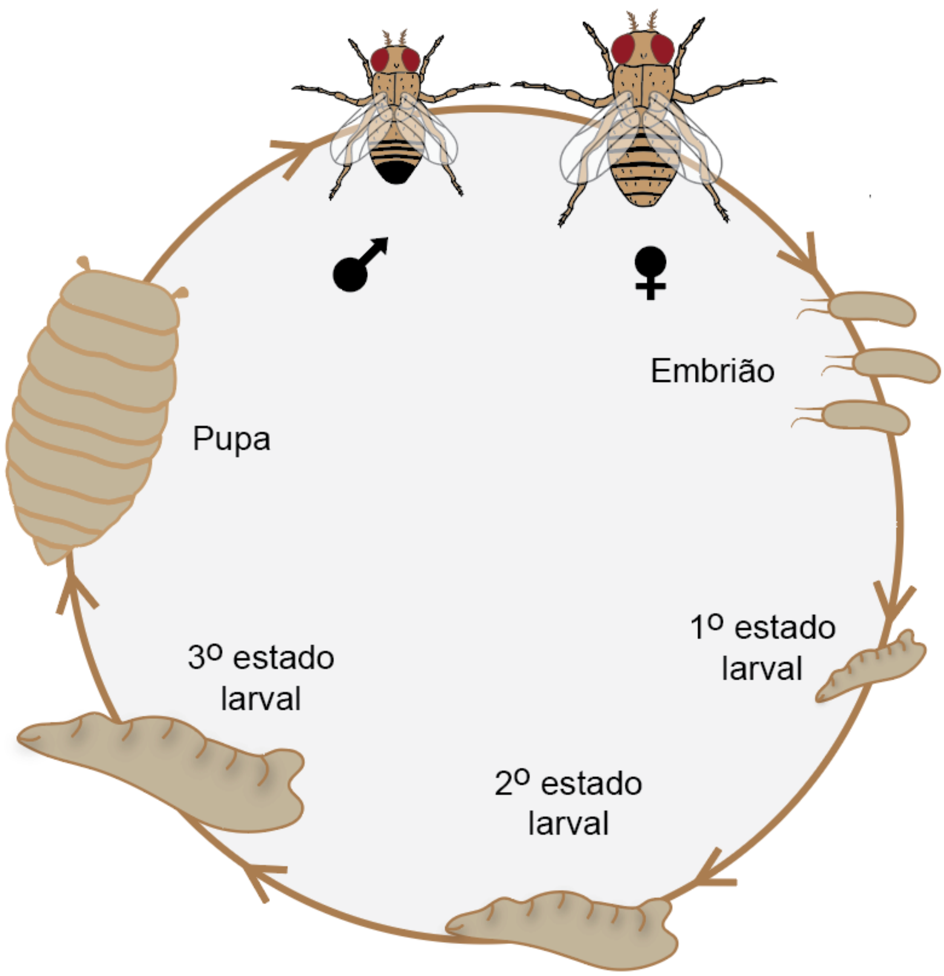


Figura 18: Ciclo de vida da *D. melanogaster* e dimorfismo sexual de machos e fêmeas.

O metabolismo é altamente conservado em *D. melanogaster*, sendo um objeto interessante no estudo de doenças metabólicas relacionadas a modelos similares à obesidade, problemas cardiovasculares e diabetes (Baenas; Wagner, 2022; de Paula *et al.*, 2018; Rivera *et al.*, 2019). A *D. melanogaster* apresenta a capacidade de regular a homeostase de açúcar e hormônios relacionado à atividade lipídica, como transporte, captação e armazenamento, embora os tecidos adiposos não sejam diretamente semelhantes ao humano. Esse inseto ainda possui alguns tecidos especializados análogos aos órgãos humanos, como o fígado, pâncreas e tecido adiposo (Baenas; Wagner, 2022; Dania; Joykutty, 2020).

3.4.2 Avaliação de marcadores bioquímicos

Marcadores bioquímicos fornecem informações importantes sobre o metabolismo e estudo sobre doenças humanas, como a diabetes, distúrbios neurológicos e obesidade, por exemplo. Certos marcadores humanos possuem similaridade com os da *D. melanogaster*, possibilitando a investigação do impacto de compostos no metabolismo do animal (Baenas; Wagner, 2022; Dania; Joykutty, 2020).

A quantificação de glicose permite avaliar o metabolismo, uma vez que esta é o principal substrato energético do organismo. Essa modulação pode ocorrer através de uma alteração na dieta das moscas por meio de uma alimentação alta em carboidratos, e pode resultar em mudanças adversas no comportamento de escalada dos animais, principalmente das fêmeas, bem como na diminuição do tempo de vida (Baenas; Wagner, 2022). O consumo de NP de ouro (AuNP) altera a concentração e o glicogênio, ao passo que aumenta a produção de espécies reativas de oxigênio (ROS) já na fase larval (Raj *et al.*, 2020). Do contrário, um material de grafeno apresentou diminuição de 36% da glicose livre e redução de 57% de ROS em moscas diabéticas (Dash *et al.*, 2024). *Solanum anguivi* Lam. também foi capaz de reduzir os níveis de glicose em um modelo análogo à diabetes (Nakitto *et al.*, 2021).

Os triglicerídeos são marcadores de homeostase lipídica, e indicam alterações na síntese, armazenamento e degradação de lipídios. Baenas e colaboradores (2022) observaram que uma dieta rica em gorduras pode alterar o comportamento dos animais, diminuindo o tempo de vida, e modulando a sinalização dos peptídeos similares à insulina. A ezetimiba, que atua inibindo a absorção do colesterol e triglicerídeos, foi capaz de diminuir, após 48h, os níveis de triglicerídeos em *D. melanogaster* em um modelo de doença esteatótica não alcoólica do fígado (Dania; Joykutty, 2020;). A infecção pela bactéria *Wolbachia* eleva o perfil lipídico e de triglicerídeos, além de reduzir expressão do gene *bmm*, responsável pela expressão da lipase lipoproteica (Karpova *et al.*, 2023)

A colinesterase está envolvida na degradação da acetilcolina no cérebro e, em *D. melanogaster*, está associada a estudos de toxicidade em pesticidas e corantes têxteis. As colinesterases são uma família de enzimas responsáveis pela quebra de colinas, como o neurotransmissor acetilcetilcolina. Essa família de enzimas inclui a acetilcolinesterase (AChE), e a butirilcolinesterase (BChE). A AChE é uma enzima de grande importância no sistema nervoso, pois catalisa a reação de quebra da acetilcolina em colina e acetato, processo essencial no final da transmissão sináptica para a regulação correta do sinal nervoso. É utilizada também na contração muscular e nas funções cognitivas; portanto, sua disfunção está relacionada com doenças neurodegenerativas, como a doença de Alzheimer e de Parkinson. A BChE é a segunda enzima da família, desempenhando funções complementares à AChE, sendo responsável pela detoxificação de xenobióticos e, ao contrário da AChE, é muito mais distribuída pelo organismo, incluindo o plasma sanguíneo. A BChE pode ainda compensar a atividade da AChE quando esta é disfuncional (De A Cavalcante *et al.*, 2018; Tsim; Soreq, 2013).

Em *D. melanogaster*, as larvas do inseto apresentaram uma maior atividade da colinesterase quando em contato com tintas têxteis, como a Disperse black-9 a 100 mg/mL (1,8 vezes mais) e 4,4 vezes mais quando exposta ao Disperse blue-124 na mesma concentração (Rahimi; Singh; Gupta, 2022). A administração de *Curcuma* não apenas reduziu a atividade de AChE em *D. melanogaster*, mas também reduziu os níveis de mRNA dessa enzima, sugerindo um potencial efeito antienvelhecimento (Akinyemi *et al.*, 2018). Os extratos das plantas *Lasianthera africana* e *Gnetum africanum* demonstraram um comportamento dose-dependente (0 a 1,154 mg/ml) com a inibição da atividade da AChE (Oboh *et al.*, 2023). *Pleurotus ostreatus* e *Lentinus subnudus* também foram capazes de reduzir a AChE, e a BChE, especialmente com 1% e 5% dos extratos adicionados à dieta (Agunloye; Oboh; Falade, 2021).

A avaliação de marcadores bioquímicos como a glicose, os triglicerídeos e a colinesterase em *D. melanogaster* mostram o potencial deste inseto no estudo de processos bioquímicos. Aliado à simplicidade e rápida reprodução, este modelo se torna indispensável para os estudos preliminares na pesquisa de novos compostos e materiais.

3.4.3 Papel do estresse oxidativo no metabolismo e a influência de materiais exógenos

O estresse oxidativo (OS) é um desequilíbrio entre as espécies reativas (RS) e a capacidade de neutralização do sistema antioxidante do organismo (Páez; Becerra; Albesa, 2011)). As RS são moléculas reativas que possuem ou não um ou mais elétrons desemparelhados na sua última camada eletrônica, e são fisiologicamente formadas em quantidades adequadas para a homeostase. Dentre as espécies principais de RS estão o radical hidroxila (·OH), sendo uma das mais reativas, o superóxido ($O_2{\cdot}^-$), que é produzido como subproduto do metabolismo respiratório e é convertido em peróxido de hidrogênio (H_2O_2) pela enzima superóxido desmutase (SOD). Sob condições crônicas e/ou de desequilíbrio das defesas naturais, forma-se um estado pró-oxidativo que causa danos a diferentes níveis moleculares (Fasnacht; Polacek, 2021; Páez; Becerra; Albesa, 2011; Seixas *et al.*, 2021)

Wang e colaboradores (2014) estabeleceram que o OS é um dos fatores majoritários no desenvolvimento de convulsões. Akinyemi e colaboradores (2018) acrescentam ainda que outras doenças ligadas ao envelhecimento também estão relacionadas com a produção excessiva de OS. Alguns estudos disponíveis na literatura fornecem informações mais detalhadas sobre a utilização como modelo para Parkinson (Naz *et al.*, 2020), influência da microbiota(Lesperance; Broderick, 2020), Alzheimer (Tue *et al.*, 2020), e doenças renais (Dow; Simons; Romero, 2022).

A expectativa de vida de *D. melanogaster* foi acrescida pela administração do extrato alcóolico de *Cyperus rotundus* em até 70% (10 mg/ml), mesmo após a exposição dos animais ao paraquat e H_2O_2. Além disso, regulou positivamente a atividade de enzimas antioxidantes como SOD1, SOD2 e catalase (CAT) (Wongchum *et al.*, 2022). Os extratos de *G. africanum* e *L. africana* também modularam a atividade de enzimas antioxidantes (oboh), assim como *P. ostreatus* e *L. subnudus* (Agunloye; Oboh; Falade, 2021).

O tempero chili foi capaz de reduzir entre 40-60% da atividade da glutationa-*S*-transferase (GST) em fêmeas e aumentar em 37% a atividade da SOD em machos (Semaniuk *et al.*, 2022). Feng e colaboradores observaram um aumento na atividade da SOD em ambos os sexos, com uma maior prevalência para fêmeas (42.19%), ao ingerirem *Chrysanthemum morifolium* enriquecido com selênio, enquanto a CAT não aparentou diferença entre ambos.

3.4.4 Estudos em *D. melanogaster* envolvendo nanopartículas

As NP apresentam fatores interessantes para aplicação na área farmacológica como o seu tamanho, facilidade de manipulação, propriedades inertes e manipulação de superfície. No entanto, por serem muito pequenas, frequentemente acabam no meio ambiente e podem ocasionar problemas de contaminação e/ou afetarem a fauna. A utilização de *D. melanogaster* é uma proposta interessante para o entendimento desses fatores e para compreender a interação com dessas NP, tanto pelo apelo ambiental quanto pela similaridade ao genoma humana (Güneş *et al.*, 2024; Mishra; Panda, 2021)

Moscas expostas a uma concentração subletal (0.03 mg CdO NPs/mL) de NP de Cádmio (CdNP) demonstraram uma queda na fototaxia em comparação com o grupo controle; contudo, não houve diferença na atividade locomotora. Ainda, os autores observaram a influência de CdNP na síntese e reutilização de histamina (Kholy; Naggar, 2022), alterando os níveis de expressão do gene da histamina decarboxilasse (hdc), a qual é responsável pela conversão da histidina em histamina.

A exposição a NP de ZnO não influenciou no tempo de vida das moscas, mas foi capaz de afetar o comportamento de escalada, indicando uma modulação do sistema locomotor. A produção de ROS também aumentou em relação a quantidade de NP. Esse aumentou pode ter relação com a genotoxicidade, uma vez que se observou um aumento nos danos ao DNA (Anand *et al.*, 2017; Ng *et al.*, 2019), indicando possíveis abnormalidades em uma exposição crônica. O intestino *de D. melanogaster* também apresentou um aumento de ROS quando expostos a NP de ZnO (Ng *et al.*, 2019)

NP magnéticas de óxido de ferro (IONPs) demonstraram efeito mutagênico e recombinante em *D. melanogaster*, sendo estes eliminados quando as IONPs foram revestidas com diferentes materiais (dióxido de silício, ácido cítrico ou polietilenamina) (Güneş *et al.*, 2024). Já as NP de magnetita apresentaram redução na fecundidade de fêmeas e atraso nas transições do ciclo de vida de *D. melanogaster* (Chen *et al.*, 2015). A exposição a NP de TiO_2 demonstrou um aumento na sobrevivência de moscas adultas com uma menor quantidade de animais mortos durante o tratamento, além de um aumento na produção de larvas, indicando uma modulação na proliferação sexual. Os autores observaram, contudo, um aumento na produção de pontos brancos nos olhos das fêmeas, o que é um indicador de mutagenicidade (Sario; Silva; Gaivão, 2018).

Os estudos com *D. melanogaster* demonstram que as NP possuem efeitos diversos, a depender de sua composição, concentração e características da superfície. Esses achados reforçam a necessidade de estudos mais aprofundados para entender os mecanismos de toxicidade, visto que tendem a ser compostos inertes, mas os quais podem mostrar o comportamento contrário.

4 CAPÍTULOS

Os resultados que fazem parte desta tese de doutorado estão divididos em três capítulos e apresentados sob a forma de artigo publicado ou manuscritos, já estruturados de acordo com as regras das revistas científicas as quais foram submetidos e em língua inglesa.

4.1 Capítulo 1

Existe uma ampla literatura sobre o uso do Nb_2O_5 como revestimento para diversos materiais, como abordado durante o referencial teórico desta tese de doutorado. Diante da falta de um material compilando vários desses resultados, se percebeu a necessidade da elaboração de um artigo de revisão como o primeiro capítulo, de forma a englobar diferentes áreas biomédicas, como citotoxicidade, corrosão, atividade antimicrobiana, entre outros.

Diante dos dados obtidos, observou-se que muitos dos estudos não avaliam o Nb_2O_5 em sua forma pura. A disponibilidade de materiais sobre o Nb_2O_5 como revestimento para materiais é extensa, mas há uma lacuna quanto às modificações superficiais no próprio Nb_2O_5. Ademais, a literatura apresentou-se escassa quanto à citotoxicidade do mesmo e estudos avaliando o impacto em um modelo animal.

O artigo foi submetido à revista **BioMetals** (fator de impacto 4.1, qualis A2), indexada na Springer Nature. Este está em revisão por pares desde 10 de novembro de 2024.

Exploring the Application of Inorganic Chemistry: Niobium Oxide as a Versatile and Promising Biomaterial

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Abstract

Inorganic materials, especially metal oxides, are gaining space in healthcare advancements due to their great stability, strength, and adaptable surfaces. Metal oxides in general can provide important modifications for the work service of biomaterials, enhancing important characteristics or introducing new ones. Niobium oxide (Nb_2O_5), for example, is a ceramic material with large applications in construction and aerospace engineering. This material is also promising for medical applications, including prosthetics, due to its great corrosion resistance and biocompatibility. With the advent of the increasing number of people worldwide requiring implants, from orthodontics to coronary stents, it is necessary to evaluate new molecules capable of meeting these demands. There has been a considerable amount of literature on the utilization of Nb_2O_5 for coatings, the material's modification, and even its sole behavior as a powder only. The properties of different Nb_2O_5 materials are illustrated in this review paper, including parameters such as corrosion, cell viability, and apatite formation.

Keywords: Niobium oxide, prosthetics, corrosion, antibacterial activity, biocompatibility.

1 Introduction

The growth of biomedical science has demonstrated the importance of developing new materials that can meet the demands of healthcare. Inorganic materials, particularly metal oxides, are emerging as major participants in this area because of their chemical stability, mechanical strength, and adapted surface characteristics ((Manivasagam et al. 2010; Eduok 2020).

Metal-oxide nanostructures provide significant benefits in tissue engineering and scaffolding applications. Substances such as zinc oxide (ZnO) and iron oxide (Fe_3O_4) can be engineered to form a variety of nanostructures that closely resemble the structural organization of natural extracellular matrices. Moreover, some metal oxides have antibacterial properties, hence diminishing the risk of infection in tissue engineering applications (Zheng et al. 2021; Hancharova et al. 2024).

Niobium oxide (Nb_2O_5) presents significant potential as a material, especially in the field of medical care, with particular relevance to prosthetics. Its excellent physicochemical features, such as a high corrosion resistance rate, catalytic activity, and biocompatibility, highlight its suitability for incorporation into biological systems. Its ability to produce stable materials is suitable for use in medical implants (Eduok 2020; Safavi et al. 2022). Moreover, the versatility of niobium oxide could sustain surface modifications, allowing the material to be functionalized to improve interactions with tissues and reduce unfavorable immune reactions.

Although promising, the biomedical applications of niobium oxide remain underexplored, with most studies concentrating on industrial and photocatalytic uses (Falk et al. 2017; Ücker et al. 2021, 2022). However, increased interest in nanotechnology and biomaterials has led to new opportunities for their application within the healthcare sector. For example, the addition of Nb_2O_5 into a Ni-Cr-Mo alloy increases the corrosion resistance rate of the material (Wadullah et al. 2022). Moreover, the combination of Nb_2O_5 with other molecules, such as hydroxyapatite (HAp), can improve cell viability (Safavi et al. 2023b).

According to the World Health Organization (WHO), approximately 30 million individuals worldwide require prosthetic and orthotic devices, with projections indicating an increase to 40 million by 2030 (WORLD HEALTH ORGANIZATION 2017). The study and use of promising metal oxides can help improve the work service and reduce the risk of rejection (Al-Shalawi et al. 2023). In light of these topics, this review paper will focus on the examination of biomedical applications of niobium oxide in relation to different important biomedical parameters, such as corrosion, cytotoxicity, apatite formation, and antimicrobial activity from current literature.

2 Materials and methods

Literature research was performed in different research websites (PubMed, Consensus, SciSpace, Google Academic, and ResearchRabbit) with the following keywords, combined or not: “niobium oxide”, “niobium pentoxide”, “Nb2O5”, “cytotoxicity”, “antimicrobial activity”, “antibacterial activity”, “inflammation”, “in vivo”, and “biomedical application”. Images were generated using VESTA (Version 3.90.0a) and Inkscape (Version 1.2.2).

3 Niobium: the element

Charles Hachett first referred to Niobium as Columbium in 1802 (Hachett 1832), a reference to Columbia, an old and fancy name for the United States of America. Heinrich Rose, a German chemist, later renamed it. Niobium was named after the mythological goddess Niobe, the daughter of Tantalus. It is found in nature in the form of minerals (Fe, Mn) M_2O_6 (M = Nb, Ta), along with other metals, such as tantalum (Ta) (Nowak and Ziolek 1999). In the 1950s, IUPAC officially named this metal niobium (Rayner-Canham and Zheng 2008)

With the discovery of pyrochlore fields (Na,Ca)2Nb_2O_6(OH,F), the Nb market value changed abruptly as it was only extracted previously from tantalite and columbite. Brazil has the largest Nb reserves (> 98%) in the world, making it the largest producer of Nb (> 95%). The *Companhia Brasileira de Metalurgia e Mineração* (CBMM) and the CMOC Group control near 80% of the total Nb market share, with the first company being the world leader (Gałaś et al. 2024). The largest Nb consumer is Europe. Additionally, Nb can be sold in different forms, such as niobium chloride ($NbCl_5$), niobium pentoxide (Nb_2O_5), and iron-niobium alloy (FeNb), among others. The first molecule can only be sold by Russia, and the most consumed material worldwide is the FeNb alloy (Safavi et al. 2022; Gałaś et al. 2024).

The steel industry reallocates almost 90% of the Nb market. Its total market value can vary annually because of global demand and production costs. The Tantalum-Niobium International Study Center (TIC) describes that Nb does not have a commodity value due to its non-trading on a metal exchange. Between 2000 and 2010, the global demand for Nb increased by 10% annually. In 2007, Brazilian production reached 82 kt of Nb, with an average demand of around 105 kt. From 2010 to 2011, FeNb trade increased by 1.3x million (Alves and Dos Reis Coutinho 2015; Bakry et al. 2022; Safavi et al. 2022; Gałaś et al. 2024).

Chemically, Niobium (Nb) is a transition metal with atomic number 41, an atomic mass of 92.906 u, and does not occur in the free state in nature. When pure, it is malleable and ductile; however, when associated with other compounds, Nb can increase the hardness of a material. Iron (Fe)- and nickel (Ni)-niobium alloys, largely used in the construction, automotive, and aerospace industries (Gałaś et al. 2024) are one of many examples, as they can reduce the carbon content in steel and thus decrease granular corrosion (Nico et al. 2016). The melting and boiling points of Nb are 2447 °C and 4741 °C, respectively (Table 1). It has a density of 8.57 g/cm^3, is a refractory metal, and is a favorable thermal conductor. The crystalline structure of Nb is a body-centered cubic (BCC) crystal lattice, similar to that of NaCl (Fig. 1). (Nowak and Ziolek 1999; Alves and Dos Reis Coutinho 2015; Safavi et al. 2022)

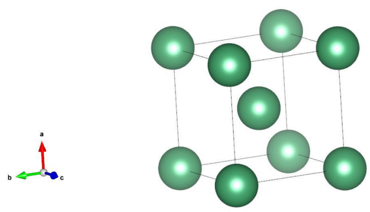

Fig. 1: Body-centered cubic (BCC) of Nb.

Nb also possesses a high affinity for oxygen, creating different oxides. This element can affect different properties of Nb, such as increasing the resistivity and changing the surface area. When in contact with oxygen, Nb has oxidation states of +II, +IV, and +V. The main oxides formed by niobium are niobium monoxide (NbO), niobium dioxide (NbO_2), and niobium pentoxide (Nb_2O_5), respectively, for each oxidation state. Among these, Nb_2O_5 is the most stable, known, and studied oxide among the reported oxides

(Valencia-Balvín et al. 2014; Nico et al. 2016; García-López et al. 2024). Nb_2O_5 is an n-type semiconductor with a band gap of approximately 3.4 eV, which is lower than that of other oxides and similar to that of zinc oxide (ZnO) (Silva et al. 2019). The interest in investigating Nb_2O_5 is due to its remarkable physicochemical properties (Raba et al. 2016). The following section presents a better overview of Nb_2O_5.

Table 1 Nb properties

Property	**Value**
Atomic numver	41
Atomic mass	92.906 u
Melting point	2447 oC
Boiling point	4741 oC
Density	8.57 g/cm3
Oxidation states	- I, 0, + I, + II, + III, + IV, + V

4 Niobium oxide

Niobium oxides (Nb_XO_Y) have relevant applications in various fields, such as catalysis, highly sensitive sensors, photo/electrochemical luminescent devices, optical fibers, batteries, solar cells, and biomedical applications. Niobium oxides can form thin films, porous nanoparticles, nanofibers, and nanowires in these applications (Yan et al. 2016; Aalling-Frederiksen et al. 2021).

Nb_2O_5 is a white powder, air-stable, insoluble in most solutions, and attacked mostly by hydrofluoric acid. Its interesting semiconducting properties and the development of more sophisticated methods of preparation have allowed it to obtain highly porous materials, very fine powders, and coatings. These novel materials have important applications in the fields of batteries, solar cells, and catalysis (Jose et al. 2009; Hashemzadeh et al. 2015; Kumar et al. 2018). The photocatalytic transformation of organic compounds and the photodecomposition of water are also excellent applications for Nb_2O_5 (Ambreen et al. 2021; Taher et al. 2021).

Structurally, Nb_2O_5 presents a great polymorphism and, therefore, generates different phases, defects, and distinct structures, which directly influence its properties and final applications. The crystalline structure of Nb_2O_5 adopts NbO_6 octahedra, which can be distorted based on each polymorphism. Hence, Nb_2O_5 crystallizes in various ways. At lower temperatures, Nb_2O_5 adopts a pseudohexagonal structure known as TT-Nb_2O_5 (Fig. 2), which the literature describes as less developed than T-Nb_2O_5 (orthorhombic) (Fig. 3). This is because TT-Nb_2O_5 requires larger vacancies in its crystal net to maintain its stability. Anions such as OH^- and Cl^- can occupy these free spaces, leading to a higher level of impurities in the crystal. It is difficult to define a specific temperature range for the crystallization changes, as there are some factors, such as time, type of synthesis, and pressure, that can influence the final Nb_2O_5 product (Nowak and Ziolek 1999; Valencia-Balvín et al. 2014; Nico et al. 2016).

The acronyms for crystalline structures come from German words (Nowak and Ziolek 1999): TT means Tief-Tief, meaning low-low, T stands for Tief (low), M means Medium, and H means Hoch (high). Some other Nb_2O_5 polymorphs received their names based on the shape of the particles, also in German: Blättter, leaves; B, N (Nadeln, needles); and R (Prismen, prisms). However, as stated by (Nico et al. 2016), there has not been an agreement among authors on the acronyms used, as some researchers prefer to use a letter according to crystallinity: T- (tetragonal), H- (hexagonal), O- (orthorhombic), and M- (monoclinic)(Fig. 4).

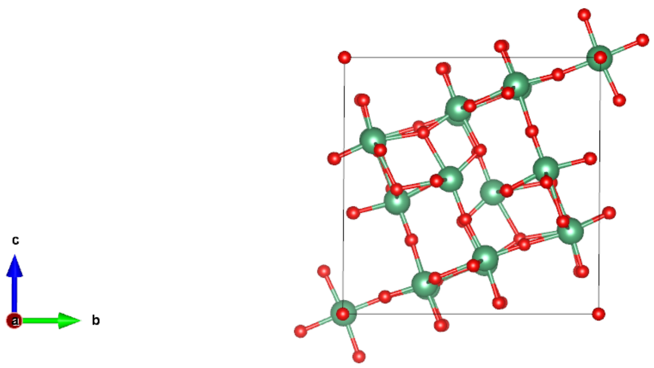

Fig 2: Nb2O5 crystalline pseudohexagonal structure.

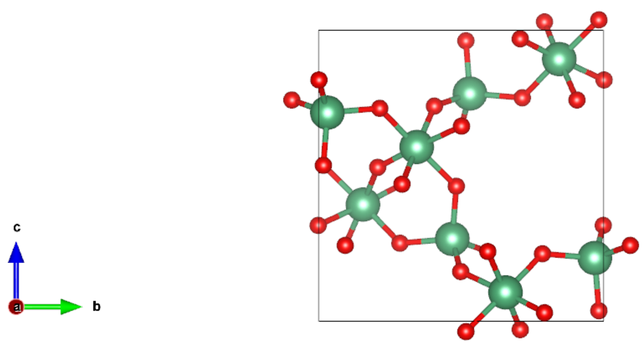

Fig 3: Nb2O5 crystalline orthorhombic structure

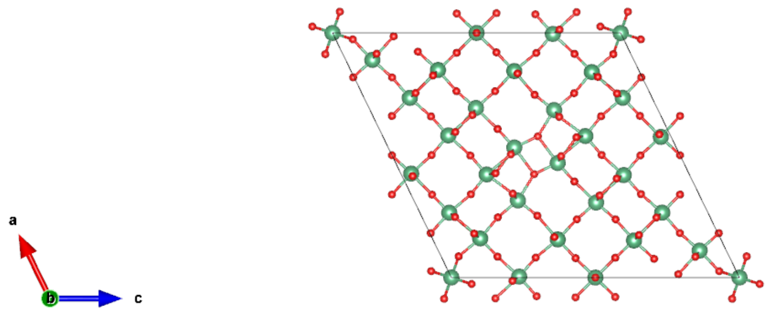

Fig 4. Nb2O5 crystalline monoclinic structure

Synthesis Methods of Nb_2O_5

In recent years, numerous studies have focused on developing efficient synthesis methods to obtain materials with controlled sizes, morphologies, and structures, which are the determining factors in their efficiency and applicability of these materials. This study presents some of the main methods used to obtain niobium oxide.

Sol-gel method

The first report on the use of the sol-gel methodology in the synthesis of Nb_2O_5 dates to the 1980s (Alquier et al. 1986). One of the methods described involved dissolving niobium (I) chloride (NbCl) in water, optionally incorporating hydrogen peroxide (H_2O_2). Subsequently, several variants of this process have been developed (Ristić et al. 2004; Granados et al. 2017).

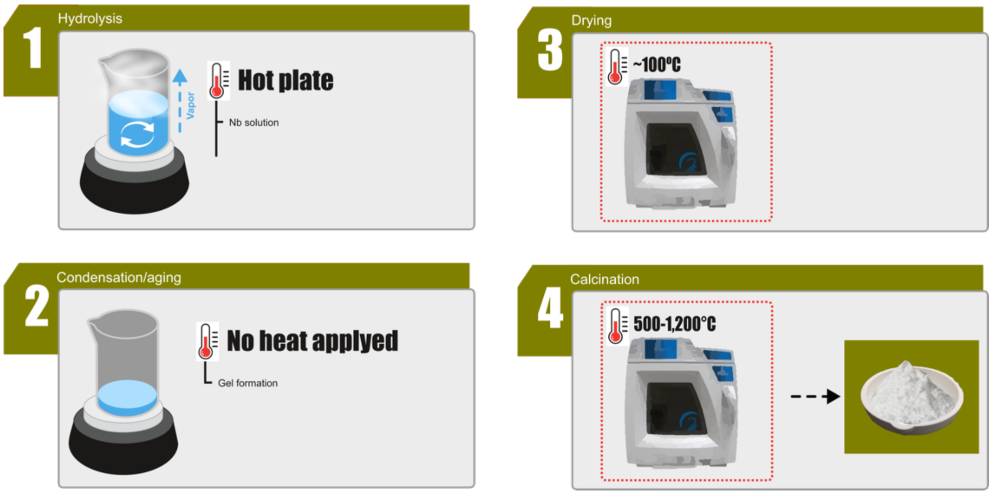

Fig. 5: Production of Nb_2O_5 using the sol-gel method.

In this method, a colloidal suspension (Fig. 5), named sol (Benvenutti et al. 2009), gelates to form a solid phase, named gel. A heat treatment is applied to remove synthesis residues, stabilize the gel, and densify/crystallize (Livage et al., 2008; Hench and West, 1990). The transition from the sol state to the gel state is accompanied by significant changes in the physical and chemical properties of the system (Ristić et al. 2004).

This process allows the production of high-purity and homogeneous materials. The sol-gel method facilitates control over stoichiometric, porosity, crystalline structure, and particle size. All of which are factors that influence the optical, mechanical, magnetic, photocatalytic, and biological properties of the final product (Lopes et al. 2015). However, one of the negative aspects of this methodology is its lengthy synthesis time. Zhou et al., (2008) reported the synthesis of Nb_2O_5 nanoparticles by the decomposition of niobic acid ($HNbO_3$) using a precipitation reaction in hydrofluoric acid (HF) and ammonia (NH_2) in aqueous solutions. At lower concentrations, they found that the Nb_2O_5 nanoparticles were between 30 and 50 nm in size.

The polymeric precursor method

The polymeric precursor method, also known as the Pechini method, is a chemistry synthesis method in which the general idea is to distribute atomic cations through all polymeric structures. The Pechini route requires the formation of chelates between metal ions and carboxylic acids (Fig. 6). These are then polymerized by a reaction with polyalcohols, and when subjected at moderate temperatures, it promotes the condensation reaction, causing polyesterification to occur (Raba et al. 2016). Thermal treatments are performed to eliminate organic matter and crystallize the material (Maciel et al. 2003).

Fig 6: Generic graphical methodology of the Pechini method.

Hydrothermal method

This method is based on simple or heterogeneous reactions in the presence of aqueous solvents under high-pressure and heating conditions that allow the dissolution and recrystallization of materials. A crystal formation is produced within a closed reactor that holds an aqueous solution that is heated above its boiling point (Shi et al. 2013). Costa et al., 2024 synthesized Nb_2O_5nanoparticles via a hydrothermal method using ammonium niobate oxalate ($C_4H_4NNbO_9$) and urea at 150 ºC for 24 h and 48 h. However, some of these reactions can take up to 96 h or more to complete the synthesis (Leite et al. 2006), which makes them less attractive.

Microwave-Assisted Hydrothermal Synthesis

Microwave-assisted hydrothermal synthesis offers many advantages. These include high reaction temperatures through the combination of microwave radiation in closed systems, reduced reaction time, high yields, fewer impurities that facilitate the purification processes, the possibility of using low-boiling point solvents under pressure and high temperatures (above the boiling point of the solvent), and the combination of microwave radiation with catalysts that absorb the radiation (Corradi et al. 2005; Wu et al. 2005).

The hydrothermal synthesis of Nb_2O_5 using the microwave-assisted hydrothermal method results in a considerable reduction in reaction time. In the studies carried out by Falk et al. 2017, the synthesis started from niobium (V) chloride ($NbCl_5$) in glacial acetic acid; subsequently, the obtained solution was hydrolyzed in a mixture of water and nitric acid (HNO_3). A commercial microwave digestion system with a frequency of 2.45 GHz was used. The heating ramp was 10 min, and the reaction time was 20 min at a maximum temperature of 180 °C. This method has numerous advantages, mainly because it reduces the synthesis time from days to hours or minutes, but it also has problems. For example, microwave reactors are very expensive, and microwaves can only go a short way through liquids. This limits the size of the reactors, making the transition to an industrial setting challenging (Bilecka and Niederberger 2010).

Green Synthesis

Green methods to produce Nb nanoparticles (NbNPs) are affordable and energy efficient. Biomolecules, such as enzymes and amino acids from plant extracts, can function as stabilizing and reducing agents (Yatish et al., 2020). Pawar et al. (2023) produced Nb_2O_5 at different calcination temperatures (450 °C and 500 °C) from Aloe Vera leaf pulp, which was confirmed by a series of characterization techniques.

Biomedical applications

Biomaterials have been engineered to interact with biological systems. Biomaterials can serve as both a therapeutic agent (drug delivery, tissue, and bone replacement) and in diagnostics. Biomaterials can be either natural (living tissues) or synthetic (ceramics and metals). They are used in various applications such as cardiovascular implants, shoulder prosthetics, disc replacement, dental implants, and bone fixation (Eliaz 2019; Menagadevi et al. 2024). According to data obtained by (Safavi et al. 2022), the coronary stent market, for example, is estimated to reach more than USD 15 billion by this year.

Prosthetics have been used since before Christ (b.C.), as the Greeks and Egyptians would use wood and animal bones in the human body and gold (Au) and iron (Fe) in dental procedures (Manivasagam et al. 2010; Eliaz 2019). Au plates were introduced in modern medicine in the 1500s and vanadium steel in the 20th century, along with the well-known 316 L Stainless Steel (SS), an alloy made of copper, nickel, and molybdenum (Co-Ni-Mo).

Besides the donor's health and medical work, a biomaterial must possess two important factors. The first is to have enough mechanical strength to hold the body and sustain its movements, as well as to be resistant to corrosion. The second factor is based on biological features: the implant should not trigger allergies, inflammation, or release metal ions into the body, thereby minimizing disruption to body homeostasis. To some extent, no material is completely inert; however, different materials possess a unique level of inertness. All these conditions must also remain stable for long periods of service (Manivasagam et al. 2010; Sukaryo et al. 2016; Sowa and Simka 2018)

In this sense, nanotechnology (the study of materials and devices ranging from 1–100 nm in spatial dimension) is of growing interest in several academic fields, such as physics, medicine, and chemistry. The word "nano" has its background in Greek, meaning dwarf (Loos 2015). The term "nanotechnology" was first adopted by Richard Feynann, a Nobel Prize winner, in the late 1950s when mentioning the possibility of creating nanoscale machines (Feynman 1959). Metallic nanoparticles have been the subject of several studies in the biomedical field, as they possess interesting features such as morphology, ease of size adjustment, and inert properties, which make them suitable for applications in the human body. Cancer immunotherapy (Evans et al. 2018) is an area of significant interest, as well as the promising cancer vaccine (Sun et al. 2024), along with the well-known study of coatings for prosthetics (Ramírez et al. 2011; de Almeida Bino et al. 2021; Moreto et al. 2021).

Metals have been used for various purposes since before the time of Christ (b.C.). According to Sreeprasad and Pradeep (2013), Egyptians used Au for spiritual purification, and the Indian medicine Ayurveda has Au in several preparations, that is, Saraswatharishtam, for memory enhancement. The Lycurgus cup, a Roman antique, contains 66.2% silver, 31.2% gold, and 2.6% copper nanoparticles, which give the glassware a unique color when reflected or transmitted with light, a phenomenon known as dichroism. The glass is on exhibition at the British Museum, and it shows King Lycurgus being pushed by Ambrosia to the underworld (Loos 2015).

A biomaterial can be classified based on its constituent material: metallic, such as nickel-titanium (NiTi) and SS; ceramic; polymeric; composites, which are a mixture of ceramic and polymeric, for example, and natural, such as skin (Safavi et al. 2022; Chong et al. 2023). There has been a shift in the literature focusing on substitutes for commercially available metals used in medicine, not only to reduce costs but also to enhance the material's work service. The high corrosion resistance and mechanical strength of niobium oxide make it an interesting option for orthopedic applications. Studies have also demonstrated its ability to promote cell adhesion and proliferation (Ramírez et al. 2011; Young et al. 2014), which will be further explored in the following sections.

In the 1970s, the total niobium content in the human body was evaluated to approximately 100 mg. Moreover, that it has affinity for the fat, liver, spleen, and other organs (Easterly and Shank 1977), but it does not indicate any toxicity (acute or chronic). (Berry et al. 1993) showed that Nb ions were found in lysosomes through co-precipitation with phosphates using radioactive ^{95}Nb. The presence of this organelle was proposed to occur through the involvement of an enzyme, acid phosphatase, which hydrolyzes phosphates in acidic environments (Anand and Srivastava 2012)

Corrosion

The human body is in a hostile environment. Electrolytes, which are important for metabolism, osmolarity, and membrane potential, can interact with metal implants and reduce their service time. Corrosion is a characteristic that determines the usefulness and service time of a material, particularly for biomedical applications (Wadullah et al. 2022). Corrosion is a natural process influenced by geometric, mechanical, and chemical characteristics that lead to material deterioration; hence, it should be regarded as the corrosion rate rather than corrosion prevention (Eliaz 2019)

There are a few solutions that can mimic different body fluids, such as Simulated Body Fluids (SBF), including artificial saliva, phosphate-buffered saline (PBS), and Hank's solution; the latter has more salts than PBS, such as sodium bicarbonate and calcium chloride. PBS appears to maintain pH stability for longer periods (Kokubo and Takadama 2006). The *in vitro* evaluation through immersion of the studied material is important, as researchers can evaluate the corrosion behavior and biocompatibility of the material before any clinical trials.

Some materials can release allergenic/carcinogenic ions (Ni, Cr, Al, etc.) when they react with different body fluids (Asri et al. 2017). Metallosis is the process by which metal implants injure and destroy hard and soft tissues. Ionic metals alone do not pose any harm, and certain metals, such as iron, which is essential for the formation of hemoglobin, can cause DNA damage at higher concentrations. Proteins and lipids from extracellular fluids (ECFs) can interact with the surface of the material and alter its properties in several ways, including (1) binding to metal ions and disrupting protein activity; (2) limitation on oxygen transportation if the protein binds to the surface of the material; and (3) creating a barrier onto the surface to avoid corrosion (Angelé-Martínez et al. 2014; Mollet et al. 2016; Permyakov 2021). Nb_2O_5 interacts with Bovine Serum Albumin (BSA) through small modifications in the secondary and tertiary structure and changes in polarity for Human Serum Albumin (HSA) around the fluorophore residues (Millan et al. 2023). The corrosion process can also loosen the implant, as it will not be fitted (Gilbert 2017; Ude et al. 2021)

The surface of the material is the first place of contact with body parts and fluids; hence, it is necessary to understand its activity. Coating is an important surface modification method for preventing the rapid occurrence of corrosion (Asri et al. 2017). Metal oxides are normally used for this purpose as they are naturally resistant to corrosion. They are also inert, which makes them suitable for biological implants. Nb_2O_5 is being studied as a coating material for its high surface area, and its polymorphism allows different properties depending on the final purpose (medical, nuclear, energetic), especially for its high corrosion rate.

Metallic implants, such as Ti_6Al_4V and 316L Stainless Steel (SS), have greater corrosion resistance when coated with Nb_2O_5, probably because Nb cations can interact with vacancies caused by Ti, for example (Al-Mobarak et al. 2011). In that sense, the Ti_6Al_7Nb started to be an alternative to the traditional Ti_6Al_4V after the 1980's, not only for being more cost-efficient but also because it removes the release of oxidized vanadium (IV) oxide (VO_2) and subsequent vanadium (V) ions to the blood flow, which could be toxic to the organism (Eliaz 2019; Al-Shalawi et al. 2023). The new alloy with Nb was found to be better for biomedical implants by possessing higher tensile strength and strain to fracture, as well as better cell proliferation and attachment in mouse preosteoblast MC3T3-E1 cells (Challa et al. 2013), as well as interaction with some important proteins such as fibronectin, vinculin, and actin.

For instance, in the case of 316L, the Nb_2O_5 layer acts as a barrier to prevent oxidation in the SBF medium (Nagarajan et al. 2010). This protection is pH-dependent: there are a few anions, such as fluoride (F^-), that are more susceptible to initiating the corrosion process when higher than 30 ppm (Nakagawa et al. 1999). (Pauline and Rajendran 2014) also showed that the deposition of Nb_2O_5 film in 316L creates a protective barrier, potentially due to the nanocrystalline structure of the Nb coating, resulting in corrosion potentials of -252 mV for the SS alone and -202 mV for the coated-SS mV.

The addition of the Nb_2O_5 (sintered at 500 ^oC) layer using a dip coating technique in 316L showed that the values for corrosion rate (CR) of the coated and non-coated 316L also on SBF immersion were 1.8x10⁻2 mmpy and 0.059x10⁻2 mmpy, respectively (Nagarajan et al. 2010). This work agrees with (Wadullah et al. 2022), even with different material and technique. The authors added Nb_2O_5 to the surface (at 600 ^oC) of a Ni-Cr-Mo alloy via a hydrothermal technique. There was an interesting improvement in the CR in SBF at 37 ^oC of the Nb_2O_5-coated material (4.02 x 10⁻3 mpy) compared to the non-coated material (6.3 x 10⁻1 mpy). The coated one also showed a lower surface roughness (4.44 nm for uncoated and 1.88 nm for the coated), which indicates that the material was tightly adherent to the surface.

A 105 nm Nb_2O_5 nanofibers (dried at 220 ^oC using the sol-gel method) along with a hybrid material (niobium oxide/acrylate) to coat carbon steel Q235 could be used as a filler to make it stronger (Eduok 2020). In that sense, Nb_2O_5 was able to increase the corrosion resistance (0.6 mg Nb_2O_5/20 g) with a coating suspension (0.01 μA cm^2) in a 3.5 wt% NaCl solution at 30 ^oC. However, this resistance decreases with higher concentrations as it leads to oversaturation of the coating.

Magnesium alloys, also known as AZ31, are also a significant metallic material. The addition of Nb_2O_5 significantly improved the corrosion resistance of AZ31, as evidenced by the significant mass loss percentage difference, with the coated material exhibiting nearly 0% corrosion after 7 days. Moreover, the uncoated material released 7-fold more Mg^2+ ions, as well as Ca (1.4-fold) and P (1.4-fold) (Amaravathy et al. 2014). Rajan et al. (2022) agrees with the previous study, also demonstrating that the Nb_2O_5-coated AZ31B showed a higher corrosion potential (values for this study were not added, as the supplementary material couldn't be accessed).

Safavi et al. (2023) evaluated a hydroxyapatite (HAp)-Nb_2O_5 coating on a nickel-titanium (NiTi) alloy and found that its addition on the surface allowed for better corrosion resistance, maybe due to the formation of an extra layer, along with the prevention of Ni leaching. Researchers found that the optimal concentration for biomineralization and improved uptake of Na^+ and Mg^{2+} in SBF was 0.5 g/L. It also had the smoothest surface (69.7 ± 1.8 nm) of all the concentrations that were tested.

Although not a corrosion process *per se*, the addition of Nb_2O_5 (sintered at 800 ^oC) and HAp in a poly-caprolactone (PCL)-gelatin scaffold showed that the increase in Nb_2O_5 concentration (up to 10% wt) also generated a more stable structure, probably because the particle interaction does not allow a higher PBS flow through the material (Marins et al. 2019).

Apatite

The ability to induce formation of apatite/hydroxyapatite (HAp) on the surface is a parameter of an active biomaterial. If a material cannot support this formation, then it's not a good biomaterial as a bone prosthetic (Kokubo and Takadama 2006). Hydroxyapatite (HAp) is an important bioceramic ($Ca_5PO_4)_3(OH)$) as it possesses chemical resemblance to a mineral component of bones, including its ability to mimic the rigidity of natural bones due to its high calcium content (Mysore et al. 2024). The 1.67 stoichiometric ratio is the ideal value to obtain for the chemical composition between calcium and phosphorus (Ca/P). The platelike apatite crystals were observed on a Nb_2O_5 coating (powder only) immersed in SBF for 30 days for the sintered sample at 525 ^oC but not for samples sintered at 450 ^oC or 650 ^oC (Pradhan et al. 2016). This ability can be related to the differences in the crystalline arrangement of the Nb_2O_5, demonstrating that the hexagonal Nb_2O_5 is more suitable for apatite formation.

A bioglass ($10CaF_2$–10CaO–$10B_2O_3$-(60-Nb_2O_5)P_2O_5–10SrO) was also evaluated for HAp deposition in SBF in respect to its bioactivity and different % mol of Nb_2O_5. The optimal concentration appeared to be 4 mol% (Madhavi et al. 2021). (Miyazaki et al. 2001) also evaluated the apatite formation on different Nb_2O_5 crystallinities, starting with a sol-gel synthesis. The as-prepared gel, sintered at both 600 and 800 ^oC, showed apatite formation, while the gel treated at 1000 ^oC did not. This is probably due to the presence of Nb-OH, but also to their structure: the latter has a monoclinic morphology, whereas the 600 ^oC showed an amorphous structure, and the 800 ^oC is orthorhombic. (Demirkol et al. 2013) showed that adding more Nb_2O_5 (5 and 10% wt) made sheep hydroxyapatite (SHA) and commercial synthetic hydroxyapatite (CSHA) composites denser. The sintered SHA at 1000 ^oC showed an increase in microhardness from 52 to 89 hv when Nb_2O_5 was increased from 5 to 10% wt, and from 99 to 118 hv for CSHA. The presence of Hap was also identified in 316L SS coated with Nb_2O_5 (Nagarajan et al. 2010) with crystal sizes varying from 1 to 3 um and the presence of Ca and P (ratio = 1.67) within 7 days of immersion in SBF.

Marins et al. (2019) observed that the HAp-Nb_2O_5 polymer scaffold had at least a 2-fold increase in the Ca and P content in 14 days, and the addition of 10% wt Nb_2O_5 caused this content to triplicate. An increase in Ca and P content was also identified in Ti_6Al_4V coated with Nb_2O_5 (Ca/P ratio 1.5 in 7 days), with the process starting with Ca^2+ as the surface is negatively charged, and subsequently the phosphate ions would bind to calcium (Nascimento et al. 2023). When NiTi was coated with different concentrations of Nb_2O_5, an increase in apatite formation was observed, especially for 0.25 and 0.5 g/L, as they generated a larger surface area with a Ca/P ratio of 1.5 and 1.52, respectively.

However, there are other parameters involved in Nb_2O_5 bioactivity. Cell viability is another important parameter to evaluate, as the material should not lead to cytotoxicity or inflammation. Although the hexagonal-Nb_2O_5 coating induced apatite formation, it also showed a decrease in cell viability after 30 days of incubation in SBF, while the amorphous one caused little to no effect (Pradhan et al. 2016).

Cytotoxicity

Before the material can be considered as a promising implant, it's necessary to evaluate several parameters in vitro, including its cytotoxicity. This area of study investigates the relationship between the object and living cells outside a living organism. Moreover, it allows us to understand changes in morphology and on a molecular level through in vitro cell culture (Alves et al. 2016; Frisch et al. 2023). This information is essential to evaluating the viability of a material, as it helps to identify any potential adverse consequences before experimenting in living tissue. This is especially relevant for Nb_2O_5, since its chemical composition and surface properties can affect its interaction with biological systems, influencing its biocompatibility.

To the best of your knowledge, there is not enough material on the cytotoxicity of Nb_2O_5 itself. Most studies are related to the evaluation of other Nb molecules, such as $NbCl_5$ or Nb-MXene (Haley et al. 1962; Yang et al. 2021) and of this molecule as a coating material for different biomedical implants (Pradhan et al. 2016; do Nascimento et al.

2021; Safavi et al. 2023b). Table 1 displays a resumed version of all literature reviewed in this topic. Different Nb_2O_5 calcination temperatures were evaluated regarding L929 mouse fibroblast cell viability: 450 ^oC, 525 ^oC, and 650 ^oC. No significant changes were observed for the first calcination temperature, but at 525 ^oC it was reduced to 64% at day 1 ($p = 0.003$) and 68% at day 7 ($p = 0.010$). At 650 ^oC, the cell viability decreased to 70% at day 1 but increased back to 100% after 30 days (Pradhan et al. 2016).

Schardosim et al. (2022) evaluated the genotoxicity of amorphous and crystalline Nb_2O_5 nanoparticles (NINPs) on Chinese ovary cancer (CHO-K1) cell lines. The amorphous and crystalline samples were annealed at 450 ^oC and 550 ^oC, respectively. The authors evaluated their genotoxicity through the comet assay, which showed an increase in DNA damage at 53, 105, and 210 g/mL ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively) for crystalline NINPs and for the amorphous NINPs at 210 g/mL ($p < 0.01$). The same researchers also looked at how CBMN-cytome (Cyt) affected chromosome damage by looking at the nuclear division cytotoxicity index (NDCI), micronuclei (MNi), nuclear buds (NBUDs), and nucleoplasm bridges (NPBs). NDCI was reduced for both types of NINPs after 24 h, with all concentrations (6.5–53 g/mL) for the amorphous NINPs ($p < 0.001$). The crystalline NINPs only exhibited a significant difference for 26 and 53 g/mL ($p < 0.001$ and $p < 0.01$, respectively), the same concentrations that increased the NBUDs after 4 h. The amorphous material did not induce any changes in that parameter, but they did increase MNi for all concentrations ($p < 0.001$) and decreased NPBs (no p value shown). On the contrary, NPBs for the crystalline sample were increased. Therefore, the crystalline material is more prone to induced DNA damage, and it may be due to the morphological differences between the crystalline and the amorphous.

Chromosomal impairment is an important marker for DNA damage. There are different parameters that can be used to evaluate that. MNi, for example, is a marker for chromosomal fragmentation or loss. NBUDs evaluate elimination of an amplified gene or chromatin. NPBs are chromosomal markers from fractured chromosomes and rearrangement from DNA misrepair (Fenech 2007; Fenech et al. 2011; Schardosim et al. 2022).

When Nb_2O_5 is added as a coating film for a material, it shows approximately a 2-fold increase in cell proliferation (96 h) between the SS 316L alone compared to the Nb_2O_5-coated (Ramirez 2011) in cementoblastoma cells (cells that are derived from the fibrous connective tissue in the periodontal ligament). Even if the cell attachment was lower, the Nb_2O_5 coating allowed cell differentiation at a higher rate than that of SS 316L alone. This may be due to the organization of the crystalline structure, and this can influence a higher cell proliferation. However, Schardosim et al. (2023) showed that the crystalline Nb_2O_5 induced DNA damage in CHO-K1 cells. The authors do not describe the preparation of Nb_2O_5 *per se*, but it can be hypothesized that it was annealed to obtain one of the crystalline structures.

Safavi et al. (2023) demonstrated that the addition of a HAp-Nb_2O_5 (monoclinic structure) coating over NiTi increased osteoblastic (Saos-2) cell proliferation. This was observed with the alkaline phosphatase (ALP) activity in prolonged periods (up to 7 days), especially for 0.5 g/L concentration, and a decrease in cell proliferation for 1.0 g/L. Moreover, the higher surface area facilitated cell growth.

Ti6Al4V coated with a Nb_2O_5 thin film was evaluated using the VERO-CCL81 cell line (de Almeida Bino et al. 2021) with a cell viability bigger than 97%. Besides, the coated material displayed a reduction in apoptosis ($p < 0.05$) for lymphocytes, helper T cells, cytolytic T cells, and polymorphonuclear neutrophils (PMN) cells, but no reduction in monocytes. (do Nascimento et al. 2021) also evaluated the same material but using MC3T3 cells, with an increase in ALP activity up to 7 days and an increase in cell viability.

The 45S5 Bioglass® (45% SiO2, 24.5% Na2O, 24.5% CaO, and 6% P_2O_5) with the addition of Nb_2O_5 in different mol% was also investigated (Hammami et al. 2023). Human osteosarcoma cell lines (Saos-2, ATCC HTB-85) were exposed to the material in two different environments: the first one, called “non-passivated extract,” allowed the cells to be in contact with the material for 24 h at 37 ^oC, and subsequently the powder was filtered; the second, “passivated extract,” was added to the previous material and kept in an incubator for an extra 24 h. The authors reported that the latter extract demonstrated increased cell viability, with an increase in Nb_2O_5 mol% correlating with improved survival.

The $(31B_2O_3\text{-x})\text{-}20SiO\text{-}24.5Na_2O\text{-}24.5CaO\text{-x}Nb_2O_5$ glass filled with different Nb_2O_5 concentrations was also evaluated for their cytotoxicity in osteoblast cells (MG63). Although the material didn’t stimulate cell proliferation, it was still non-toxic according to the MTT assay, as they didn’t have any significant statistical difference ($p > 0.05$) with the control in 48 h (Samudrala et al. 2018). On the contrary, the hybrid material comprising Nb_2O_5 and polydimethylsiloxane (PDMS) was able to induce cell proliferation in primary human fibroblasts, with a higher increase (approximately 5-fold) in 40 Nb_2O_5 wt% after 3 days (Young et al. 2014).

A phosphate inverted glass ($60CaO$-$30P_2O_5$-$(10-x)Na_2O$-xNb_2O_5) is another glass used in medical and dental areas that can be used as bone filler that can be shaped according to necessity (Rahaman et al. 2011). The addition of 3% and 5% mol of Nb_2O_5 ($p < 0.05$) indicated an increase in ALP activity in the MC3T3-E1 cell line (Obata et al. 2012). A "medium extract" containing only Nb traces was also evaluated with the 3.44 $\times 10^7$ M Nb ions reaching the peak at day 14 ($p > 0.05$) with no osteogenic factors (ascorbic acid, β-glycerophosphate, and dexamethasone), but at day 7 in the presence of them ($p > 0.05$).

Cell adhesion is another important factor when evaluating biocompatibility. If a material can promote cell attachment and spreading, the integration with the host tissues is successful (Aymerich et al. 2017). The interactions between the surface material and the cells must be successful so that the material won't be rejected by the organism. In that sense, (E. Eisenbarth, D. Velten, M. Muller, R. Thull 2006) evaluated different Nb_2O_5 coatings' roughness (Ra = 7 nm, calcined at 450 ^oC, quasi-amorphous; Ra = 15 nm, calcined at 500 ^oC, monoclinic; Ra = 40 nm, calcined at 700 ^oC, monoclinic) for commercially pure (CP) Ti in osteoblast-like (MC2T2-E1) cells. The authors found that cells attach initially (t = 15 min) better on smoother surfaces (Ra = 7 nm), as well as cell migration, but no different effect was observed after 90 min, indicating that the initial attachment depends on the type of surface. In agreement, the roughest coating (Ra = 40 nm) also negatively influenced cell spreading.

Interestingly, the same authors showed that collagen-I synthesis, an important factor for bone remodeling around the implant, was incomplete for the smoother surface (Ra = 7 nm) but better for the intermediate (Ra = 15 nm) coating (E. Eisenbarth, D. Velten, M. Muller, R. Thull 2006). The bone attachment to the implant is initially regulated by the surface, this one serving as a connection between both. (Li et al. 2016) didn't find a cell proliferation increase in Saos-2 when adding Nb_2O_5 to pure Ti. The mixed material, sintered at 1000 ^oC, was, however, non-toxic. This may be due to the differences in the material's structure and synthesis (via powder metallurgy) and/or the higher calcination temperature.

Tricalcium silicate (TCS) mixed with Nb_2O_5 (30%) was also evaluated in Saos2 (Queiroz et al. 2021) for endodontic purposes. The authors demonstrated that the extract dilutions (the material is kept in medium, and the measurement made indirectly through it) were not cytotoxic in the MTT assay, and for higher dilutions (1:18 and above), they did seem to have a slight increase in cell proliferation. For the neutral red (NR) assessment, the dilutions didn't show any statistical difference compared to the control. The material also overexpressed the ALP's mRNA, indicating an increase in osteogenic differentiation. These results agreed with Ge et al. (2013), who observed an ALP overexpression with the increase of Nb_2O_5 content, especially for 50 v% Nb_2O_5 over PEEK composites in bone mesenchymal stem cells (BMSC). The ALP content was also evaluated in a Nb_2O_5-PCL-Gelatin scaffold (Marins et al. 2019), in which the activity also increased over the course of 7 days, regardless of the amount of Nb_2O_5. The Saos-2 cell viability significantly increased from days 1 to 7 and 3 to 7, with the highest metabolism for 7 wt% Nb_2O_5 ($p < 0.05$).

The adsorption ratios of both bovine serum albumin and fibronectin increased with the content of niobium pentoxide (Nb_2O_5) in polyetheretherketone (PEEK)- Nb_2O_5 composites (Ge et al. 2013). The BSA adsorption ratios for PEEK, PNC25 (PEEK25%v Nb_2O_5), and PNC50 (PEEK-50%v Nb_2O_5) were 14.19 ± 3.10%, 27.74 ± 2.32%, and 35.86 ± 3.48%, respectively. The adsorption ratios of fibronectin were 12.20 ± 3.10%, 23.04 ± 3.10%, and 33.87 ± 4.64%, respectively. The material's roughness was also increased with the increase in Nb_2O_5 content, and the increase in protein adsorption is related to the increase in Nb content.

In A549 tumor cells, the photodynamic activity of Nb_2O_5 was evaluated along with protoporphyrin IX (PPIX) and tris(ethynylphenyl) pyrene derivative ($PyPh_3$) modifications (Oliveira et al. 2024). Cells were irradiated with LED (1.6 J cm−2) for 15 min and evaluated with MTT assay. Although the Nb_2O_5 dyes were less efficient than PPIX or $PyPh_3$ alone (IC_{50} of 4.7 ± 0.06 and 5.4 ± 0.04 μmol L^{-1}, respectively), they still had a relevant activity: Nb_2O_5-APS-PPIX (IC_{50} 13.1 ± 0.2) and Nb_2O_5 -APS-$PyPh_3$ (11.9 ± 0.02 μmol L^{-}1). The antitumor activity of Nb_2O_5 was also evaluated against L20B (a mouse transfected cell line expressing human poliovirus) and MCF7 (human breast cancer) cell lines (Salim et al. 2022), indicating a 60% growth inhibition for the first cell line at 50 μg/ml and 33.9% for the latter one.

Amaravathy et al. (2014) observed that the magnesium AZ31 alloy coated with Nb_2O_5 allowed more MG-63 osteoblast cells (7000 cells) to attach to the surface than the uncoated material (3200 cells). This could be attributed to the medium becoming alkalinized due to the release of Mg^{+2} ions.

Table 2 Most relevant cytotoxic findings on Nb_2O_5

Authors	Material	Type/Temperature of Nb	Type of deposition	Cell line	Results
Amaravathi et al., 2014	AZ31-Nb_2O_5	380 °C, monoclinic	Hybrid synthesis	MG-6	Higher cell attachment (7000 cells) that uncoated material (3200 cells)
Bino et al., 2021	Ti_6Al_4V- Nb_2O_5	Nb (99.9%)	Reactive sputtering technique	VERO-CCL-81	Cell viability up to 97%, reduction of apoptosis for lymphocytes, helper T cells, cytolytic T cells, and PMN cells
Eisenbarth et al., 2006	Nb_2O_5-CP Ti	(a) 450 °C, quasi-amorphous; (b) 500 °C, monoclinic; (c) 700 °C, monoclinic	Sol-gel	Osteoblast-like(MC2T2-E1)	Initial attachment better on quasi-amorphous; cell spreading negatively influenced by roughest coating (700 °C, monoclinic); collagen-I synthesis better for intermediate coating (500 °C, monoclinic)
Ge et al., 2013	Nb_2O_5-PEEK	550 °C	Pressing sintering	Bone mesenchymal stem cells (BMSC)	ALP overexpression with increase of Nb2O5 content, especially for 50v% $Nb_2O_5$and increase in cell adhesion rates Increase in fibronectin adsorption with increase in % Nb_2O_5
Hammami, 2023	45S5 Bioglass ®-Nb_2O_5	800 °C, amorphous	Melt quenching technique	Saos-2	Higher cell viability with passivated extract, better metabolism with increased % Nb_2O_5
Li et al., 2016	Nb_2O_5-CP Ti	1000 °C	Powder	Saos-2	No cell proliferation increase compared to the control, non-toxic when sintered at 1000°C
Marins et al., 2019	Nb_2O_5-PCL-gellatin scaffold	800 °C, orthorhombic	Electrospinning	Saos-2	Increase in ALP activity over 7 days; significant increase in cell viability; highest metabolism for 7 wt% Nb_2O_5
Nascimento et al., 2023	Ti6Al4V- Nb_2O_5	Nb (99.9%)	Reactive sputtering technique	MC3T3	Increase in ALP activity up to 7 days, increase in cell viability
Obata et al., 2012	60CaO-30P2O5-(10-x)Na2O-x Nb_2O_5	1400 °C	Conventional melt-quenching	MC3T3	Increase in ALP activity in MC3T3-E1 cell line with 3% and 5% mol of Nb_2O_5
Oliveira et al., 2024	Nb_2O_5-APS-PPIX and Nb_2O_5-APS-$PyPh_3$	75 °C, amorphous	Amidation	A549	Less efficient than PPIX or $PyPh_3$ but still relevant Nb_2O_5-APS-PPIX (IC50 13.1 ± 0.2 µmol L-1) and Nb_2O_5-APS-$PyPh_3$ (11.9 ± 0.02 µmol L-1)
Pradhan et al., 2012	Nb_2O_5	(a) 450 °C, amorphous; (b) 525°C, hexagonal (c) 650 °C, orthorhombic	N/A*	L929	No changes in cell viability for 450 °C; at 535 °C reduction to 68% at day 7; at 650 °C reduction to 70% at day 1 but recovery to 100% at day 30.

Table 2 Most relevant cytotoxic findings on Nb_2O_5

Authors	Material	Type/Temperature of Nb	Type of deposition	Cell line	Results
Queiroz et al., 2020	TCS- Nb_2O_5	Nb (99.9%)	Polymeric precursor	Saos-2	Not cytotoxic in MTT assay, slight increase in cell proliferation for higher dilutions, ALP's mRNA overexpression indicating osteogenic differentiation
Safavi et al., 2023	Nb_2O_5-HAp-NiTi	Not stated	Electrodeposition	Saos-2	Prolonged periods of ALP activity up to 7 days (0.5 g/L) Decrease cell viability for 1.0 g/L
Salim et al., 2021	Nb_2O_5	Colloidal solution, monolinic	N/A	L20B MCF7	60% growth inhibition for L20B 33.9% growth inhibition for MCF7
Samudrala et al., 2018	(31B2O3-x)-20SiO-24.5Na2O-24.5CaO-x Nb_2O_5	Nb_2O_5 (99.9%)	Melt quenching	MG-63	Non-toxic according to MTT assay, no significant difference with control in 48h
Schardosim et al., 2022	Nb_2O_5	(a) 450 oC, amorpohous, (b) 550 oC, crystalline	N/A	CHO-K1	DNA damage at 53, 105, and 210 µg/mL for crystalline, and amorphous at 210 µg/mL Amorphous material increased MNi and decreased NPBs. NDCI decrased for both.
Young et al., 2013	PDMS- Nb_2O_5	Not stated	Dip coating	Primary human fibroblast	Approximately 5-fold of cell viability increase after 3 days for 40% wt Nb_2O_5

* N/A: not applicable.

Inflammatory Response

The immune system is a defense mechanism that recruits different types of cells derived from the bone marrow (Kuwabara et al. 2017) to maintain overall health and to protect the body against infections and diseases. Corrosion, for example, can induce and/or increase inflammation due to the release of metal ions from the material's surface (Sowa and Simka 2018). This highlights the importance of developing biocompatible materials that do not cause harmful biological responses.

Moreto et al., (2021) used DC-magnetron sputtering to evaluate the inflammatory response when coating 316L with Nb_2O_5. There was a decrease in human gingival fibroblast cell line HGF-1 in the TNF-*α* and IL-17 production ($p < 0.05$), but similar levels of IL-10, INF- *α*, IL-4, and IL-6 (no significant difference). A reduction in TNF-*α* production is important for a long-term implant, as this cytokine helps regulate the inflammatory response in the body. Moreover, it's also related to osteoblast activation, as well as for IL-17, which is involved in osteoclastogenesis (Kuwabara et al. 2017) and can modulate TNF-*α* secretion (Jang et al. 2021).

Bino et al., (2021) also evaluated IL-10, IL-6, and TNF-*α* levels of exposure to Ti6Al4V coated with Nb_2O_5 (via reactive sputtering technique) in the VERO-CCL-81 cell line, indicating an increase in the production of all three inflammatory markers. Only IL-10 had a statistically significant difference ($p <0.05$).

Antibacterial activity

Antimicrobial resistance (AMR) is a natural process in which the microorganisms are exposed to active agents and no longer respond to their efficacy. For them, it is a defense mechanism to fight for their survival; for humans, otherwise, the AMR is considered one of the major public health problems worldwide. Although the AMR is a natural process, there are a few human actions that accelerate it, including the (1) excessive and incorrect use of antimicrobials, such as antibiotics; (2) overuse of these drugs in veterinary medicine and agriculture; and (3) incomplete treatments and/or improper selection of dose (Ferrara et al. 2024; Aslam et al. 2024).

There are more than 500,000 deaths registered every year because of the AMR. It is expected that in 25 years, the most common cause of death will be untreated infections, and it could surpass cancer and cardiovascular diseases. The AMR causes not only a prolonged time of treatment for acute diseases but also lifelong conditions, as is the case for immunocompromised patients (Hassoun-Kheir et al. 2020). Invasive procedures also would be affected, from a simple tooth extraction to a heart transplant, increasing the risk of infections. Furthermore, the World Bank Group (2017) estimates that if we don't address the AMR problem, over 24 million people will face extreme poverty. *Escherichia coli*, for example, one of the most common human urinary tract infections, increased its resistance to normal treatment by almost 4% from 2017 to 2020 (ONU 2022). This indicates that 20% of the total known strains of this bacteria acquired some resistance to third-generation cephalosporin.

In this regard, the urgent need for innovative antimicrobial agents and alternative therapeutic strategies becomes apparent as conventional antibiotic treatments continue to lose their effectiveness and face increasing challenges. Some studies have been demonstrating the use of different metal ions to provide antibacterial activity (Sadeghi et al. 2012; Hancharova et al. 2024; Holubnycha et al. 2024). To the best of our knowledge, there are only a few studies on the antibacterial activity of Nb_2O_5, or they were not easily accessible in the literature.

Hammami et al. (2023) investigated the antibacterial effect of 45S5 Bioglass with Nb_2O_5 addition for three different bacterial strains (*Eschericia coli* K12 DSM498, *Staphyloccus aureus* COL MRSA (methicillin-resistant strain), and *Streptococcus mutans* (DSM20523). They found that the powder showed a great potential as antibacterial, especially for Nb_2O_5 concentrations higher than 2 mol%, perhaps due to pH alteration and changes in osmotic pressure. These parameters alter the bacteria's morphologies and impact the cell membrane integrity.

Another Nb_2O_5-modified glass ($10CaF_2$–10CaO–$10B_2O_3$-(60-Nb_2O_5)P_2O_5–10SrO) showed a strong antibacterial effect against *S. aureus* and *E. coli* (no ATCC or code given by the authors) at all Nb_2O_5 concentrations tested, especially when using a 4% mol, with 30 ± 1 mm and 25 ± 1 mm inhibition zones for each bacteria. It was also observed that for *S. aureus*, smaller concentrations (1% and 2% mol) also had the same effect (Madhavi et al. 2021). This could be regarded as the highly osmotic pressure that $Nb^{+}5$ ions promote in the environment, not allowing bacterial growth. The increase in concentration from 4 to 5% decreased the antibacterial activity, probably because of a change in the crystalline structure from NbO_6 to NbO_4.

The combination of TCS + 30% Nb_2O_5 showed great performance when exposed to *Enterococcus faecalis* (ATCC 29212). Among all tested combinations, the use of Nb_2O_5 completely eradicated the bacterial count after 48 hours at 37 °C ($p < 0.0001$) (Queiroz et al. 2021). Furthermore, the addition of Nb_2O_5 and ZnO (0.5:0.5) coating in 316L was efficient in inhibiting 41% of S. aureus and 90% of E. coli. (no ATCC or code informed in the article), and it may be due to the release of reactive oxygen species (Kumar et al. 2018) However, the authors state that the presence of ZnO is primarily responsible for this behavior.

Altmann et al. (2017) conducted a study to assess the antibacterial activity against *Streptococcus mutans* (UA 159) in a new orthodontic phosphate inverted glass adhesive (75 wt% BisGMA, 25 wt% TEGDMA, 5 wt% fumed silica, and photo-initiator system TPN) containing 1,3,5-tryacryloylhexahydro-1,3,5-triazine (TAT) and Nb_2O_5 (TPN). They found that the adhesive reduced the log CFU/ml (4.22 ± 0.3) compared to the control (5.62 ± 0.16) and to a commercial adhesive (TTransbond XT, 5.51 ± 0.42). The application of antibacterial compounds in brackets is of extreme importance as it can prevent not only an infection but also maintain the stability of the orthodontic setting without any dislocation or demineralization (Salehi et al. 2018).

The Ti_6Al_4V coated with Nb_2O_5 showed that with direct contact there is an acute reduction in S. aureus CFU at 3 h but an increase after 6 h. The disk diffusion agar test revealed no antibacterial activity, potentially due to the inability of Nb ions to diffuse in the agar (Nascimento et al. 2023). It is important to mention that Staphylococcus is the main bacterial cause of orthopedic implant infections.

The use of titanium dioxide (TiO_2) is increasing in biomedical research, can be used in dental adhesives and toothpastes (Mansoor et al. 2022; Ali and Alwan 2023), and is already widely used in sunscreens, as it can block both UV-A and UV-B light (Syalsabilla et al. 2023).The incorporation of Nb_2O_5 in a TiO_2 nanopowder $80TiO_2.20 Nb_2O_5$ (Bachvarova-Nedelcheva et al. 2024) showed an interesting antibacterial effect against *E. coli* (NBIMCC K12 407) and *Bacillus subtilis* (NBIMCC 3562). The gram-positive bacteria showed a reduction of 30.2% in planktonic cell count, while *E. coli* 14.8% after 24 h of incubation for when the material was previously sintered at 200 °C. When the powder was calcined at 400 °C, a decrease in the antibacterial was observed (9.3 and 7.4%, respectively).

In vivo animal testing

While still controversial, animal testing still plays an important role in the study of vaccines, medications, and procedures before they are applied to human life. There are a few movements, such as the 3 R's, meaning reduce, reuse, and recycle, and alternatives, such as using less developed animals, like fish. However, it's an ongoing process that will take a longer time (Ferdowsian and Beck 2011; Ritskes-Hoitinga et al. 2023). The (2014)Safety data sheet does not report Nb_2O_5 as a toxic compound; however, it advises that exposure to the eyes and/or respiratory tract may cause irritation. The National Institute of Health (2014) also mentions that it may cause skin irritation, and it can be harmful to mice and rats over 4 mg/kg.

Toxicity was performed in vivo in Swiss male mice (90 days) with a 3% commercial Nb_2O_5 solution (1 mL) added intraperitoneally (Dsouki et al. 2014). The animals showed a mild degeneration of hepatocytes until day 7 and an increase in Kupffer cells, but at day 12 they displayed cell regeneration. There was also no significant hematological alteration. Although there was an increase in peritoneal cells, Nb_2O_5 was only cytotoxic in the acute inflammation process, not in cell death or changes in white blood cells.

Biphasic Calcium Phosphate (BCP) is a combination of HAp with Beta-tricalcium phosphate (-TCP) [Ca3(PO4)2] that can improve a prosthetic (Liu and Lun 2012). While HAp promotes osteoblast proliferation, -TCP provides Ca and P ions, forming a biphasic ceramic. The main disadvantage is that BCP is too fragile. The addition of Nb_2O_5 (ratio 1:1, sintered at 1000 ^{o}C) improved 17% the material's density, 66% in Vickers microhardness, and 180% in compressive strength compared to the pure BCP. Wistar rats (*Rattus norvegicus, var. albinus*) with 90 days received a critical-size bone defect into the calvaria using a trephine drill, and the Nb-BCP disk was inserted. Despite the absence of complete bone regeneration in 60 days, the bone margin showed evident growth, and blood vessels developed around the implant.

A bioactive glass, largely used in dentistry, was also evaluated for its bone healing ability when structurally modified with Nb_2O_5 (Balbinot et al. 2019), using a combination of tetraethylorthosilicate (TEOS), triethyl phosphate (TEP), calcium nitrate ($CaNO_3$), sodium nitrate ($NaNO_3$), and niobium chloride ($NbCl_5$). The material's insertion was done on the anterolateral surface in the femur of male rats (*Rattus Novegicus Albinus, Rodentia Mammalia*—Wistar lineage) after performing a small defect. The different types of material (powder or scaffold) influenced the mineralization activity, making the powder more efficient. Moreover, the Nb-modified glass displayed a relative higher volume of bone density.

5 Conclusion

Niobium oxide is a promising material that has been gaining space in the biomedical area due to their ability to modify prosthetic surfaces and improve their mechanical qualities, such as altering the corrosion rate to improve a material's work service. In summary, different Nb_2O_5 synthesis techniques and depositions were described and can be used for coupling with different materials, such as stainless steel, bioactive glasses, and dentistry adhesives. Nb_2O_5 in general can enhance the material's need, such as an increase in corrosion or reducing inflammatory markers. The result will depend on the specific choice of protocol for the synthesis and/or coating. There is still much to be investigated, especially on the study of pure Nb_2O_5 itself and its different calcination temperatures, as there is a big array of parameters to choose from. There are several other research articles about Nb_2O_5 available in the literature that were not discussed in this review, but they are also highly important for understanding this molecule. Moreover, the exact mechanisms by which Nb_2O_5 interacts with different biological systems, i.e., cell viability, antibacterial properties, and anti-inflammatory pathways, are still a long road to discover.

Acknowledgements. The authors gratefully acknowledge the financial support of the Coordination for the Improvement of Higher Education Personnel (CAPES).

Authors contribution: MMC: draft, writing, editing, translation, table creation, formatting; GCC: draft, writing, review FCR: draft, writing, image creation; PDZ: review; MPA: writing; NLVR: review, supervision; JLG: draft, review; RAV: writing, review, supervision.

Declarations

Conflict of interest

The authors declare no competing interests

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4.2 Capítulo 2

Os resultados desse capítulo são apresentados em forma de manuscrito preliminar. O segundo capítulo deste trabalho de tese buscou estudar as modificações causadas pela adição *in-situ* de surfactantes aniônicos ou catiônicos em ON, e avaliar a citotoxicidade hemolítica, hepática e a atividade anticoagulante destes compostos.

A caracterização de um material é necessária para entender e correlacionar o comportamento biológico deste. Nesse sentido, foi investigado se houve alguma modificação superficial causada pela adição de surfactantes. Ainda, estas modificações causaram diferenças nas atividades biológicas, mesmo não havendo correlação linear entre os fatores de síntese e a citotoxicidade.

Os ON são considerados, em grande parte, como não tóxicos, e inclusive permitindo o aumento da viabilidade celular em alguns casos. Contudo, dado os diferentes polimorfismos e tipo de síntese utilizada, muitas versões podem ser obtidas para um mesmo material. Dessa forma, é necessária uma triagem inicial para a compreensão do comportamento de óxidos de Nb amorfos obtidos pelo método de micro-ondas.

Alguns resultados estão ausentes em consequência da espera de laboratórios parceiros, como a análise elementar dos compostos e a espectroscopia de fotoelétrons excitados por raios X (XPS), mas serão implementados no artigo após a defesa de tese. É importante mencionar que, apesar da vasta literatura sobre a caracterização de diferentes ON, não foram encontrados dados similares para a parte biológica, tratando-se, portanto, de um estudo inédito.

O manuscrito foi submetido para publicação na revista ***Biomedical Materials***, indexada na IOP Science (fator de impacto 3.9, qualis A1) e está em fase de reestruturação após parecer positivo dos revisores

Journal **XX** (XXXX) XXXXXX https://doi.org/XXXX/XXXX

Structural, Morphological, and Cytotoxicity Evaluation of Surfactant-Modified Niobium Oxides

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Abstract

The employment of metal nanoparticles for biomedical applications is gaining visibility as a result of their excellent properties. Niobium oxide (Nb_2O_5) possesses interesting physicochemical properties that can be modified for its use in prosthetic coatings. However, there are only a limited number of studies in the literature concerning its characterization as a pure powder and its surface modification. Therefore, the purpose of this study was to evaluate nine different Nb_2O_5 samples synthesized using the microwave technique, each with a different surfactant. XRD results indicated that all samples were amorphous, and the addition of surfactants did not seem to cause any alterations, as indicated by Raman and FTIR. SEM images revealed that the particles tended to form aggregates; modification of parameters such as surface area and acid sites was also observed, with pure Nb_2O_5 having the highest area (230.4 m^2/g) and NbSDS5 having the highest total acidity (3141 μmol/g). We assessed the cytotoxicity in sheep's erythrocytes and the Zebrafish Liver (ZF-L) cell line. Pure Nb_2O_5 exhibited high cytotoxicity at 10 mg/mL in red blood cells with an erythrocyte survival rate of 15%. Compounds were able to interfere with the intrinsic coagulation pathway, with several samples exceeding the clotting time (> 120 s). Cytotoxicity was evaluated by MTT assay that revealed that NbCA1 showed only 27.1 % cell viability, while NbSDS1 was able to increase cell proliferation (101.1 %) even at a lower pH. Nb ions leaching to the medium does not seem to directly affect cytotoxicity. Pearson's correlation does not indicate a direct relationship between surface area, acid sites, and cytotoxicity assays.

Keywords: Niobium oxide, cytotoxicity, surfactants, microwave synthesis, surface modification.

1. Introduction

Biomaterials have become a vital component of modern medicine, providing solutions for a wide range of clinical applications such as tissue engineering, drug delivery, and medical implants. These materials, which can be made from metals, ceramics, polymers, or composites, are intended to interact with biological systems in a controlled way. Metallic nanoparticles (MNPs), for instance, are being studied because they possess ease of altering their size and inert qualities, which makes them suitable for a biological system (Eliaz, 2019; Manivasagam; Dhinasekaran; Rajamanickam, 2010; Sukaryo; Purnama; Hermawan, 2016).

MNPs can be synthesized from different techniques, but they mostly involve reactions with their related metal salts Several agents, such as 3-mercapto-1-propansulfonate (3MPS) and 1-thio-D-glucose (TG), can stabilize these NPs, as is the case for gold nanoparticles (Porcaro *et al.*, 2016). They altered not only the particle aggregation but also its bioactivity in radiotherapy applications. The surface functionalization of MNPs can impact the therapeutic approach of the material. This is the case for silver nanoparticles, in which the surface coating with 3MPS showed lesser toxicity than the capping with L-cysteine (Bellingeri *et al.*, 2024).

Gromnicova et al. (2013) showed that AuNPs could cross the blood-brain barrier, thus affecting neurological pathways. The capping of different materials (glass, polymers, semiconductors) with ZnNP demonstrated that its water affinity was altered (Singh; Singh, 2018). Moreover, the addition of cetyltrimethylammonium bromide (CTAB) to the synthesis of AgNPs enhanced the minimum inhibitory concentration against *Staphylococcus aureus* and *Escherichia coli* (Pisárčik *et al.*, 2018), and the addition of Triton X-100 can reduce the TiO_s toxicity in *Daphnia magna* (Oleszczuk; Jośko; Skwarek, 2015)

Niobium oxide (Nb_2O_5) has gained significant attention in the past few years due to their unique physicochemical properties and versatile applications in various industries (Nico; Monteiro; Graça, 2016a). Research on Nb_2O_5 in biomedical applications primarily focuses on its use as a coating film for prosthetics, including stainless steel 316L SS (Ramírez *et al.*, 2011), Bioglass® (Hammami *et al.*, 2023), and NiTi (Safavi; Khalil-Allafi; Visai, 2023). However, the study of Nb_2O_5 itself and its surface modification remains underexplored, which can significantly influence their interaction with biological environments.

Surface modification has emerged as an important tool for the development of new strategies to enhance the biocompatibility and functionality of different materials (Abdelkawi *et al.*, 2023). Surfactants can change the surface charge, hydrophobicity, and dispersibility of niobium oxide, thereby influencing their activity and potential cytotoxic effects (Zhang *et al.*, 2014). Moreover, to the best of our knowledge, there are only a few studies on the toxicity and genotoxicity of Nb_2O_5 pure powder. Understanding its sole behavior is important for the improvement of Nb-based biomaterials.

The goal of this study is to describe a group of surfactant-modified Nb_2O_5 and evaluate their cytotoxic effects on erythrocytes and Zebrafish Liver (ZF-L) cell line. By investigating the properties of these nanoparticles and assessing their biocompatibility, we seek to provide insights into their potential applications and safety in biomedical contexts. Our findings suggest that the samples were hemolytic at higher concentrations, and this activity may have a relationship with the surface area and the acid sites. In addition, the modified Nb_2O_5 samples displayed different behaviours, whether being cytotoxic or improving cell proliferation on ZF-L cells.

2. Materials and Methods

2.1 Materials

All chemicals used in this study were of analytical grade and did not pass through any purification before use. Niobium pentachloride ($NbCl_5$, 99.9 %), Sodium dodecyl sulphate (SDS, ≥ 99 %), Cetyltrimethylammonium bromide (CTAB, ≥ 98 %), Oleic acid (OA, > 90 %), Citric acid (CA, > 99.5 %), Hydrogen peroxide (H_2O_2, 30 % w/w aqueous solution), HCl (37 % w/w aqueous solution) and human citrated plasma were purchased from Sigma Aldrich (Missouri, USA). Phosphate-buffered saline (PBS) and defibrinated sheep's blood were purchased from Laborclin® (Paraná, Brazil). Triton X100 was acquired from Thermo Fisher® (Massachusetts, USA), and insulin (100 UI/mL) and saline solution (0.9 %) from a local pharmacy. Leibovitz's L-15 and Ham's F-12 media were acquired from Vitrocell Embriolife (São Paulo, Brazil). Dulbecco's Modified Eagle's Medium (DMEM) with high glucose content (4%) was purchased from Inlab Diagnóstica® (São Paulo, Brazil). Trypsin, Fetal Bovine Serum (FBS), and 3(4,5-dimethyl)-2-bromide-5 diphenyl tetrazolium (MTT) were obtained from Gibco® (California, United States).

2.2. Synthesis of Niobium Pentoxide Catalysts by Microwave Method

The surfactant-growth Nb_2O_5 was performed through the microwave-assisted (MW) synthesis according to Ücker *et al.* (2019) (Ücker *et al.*, 2022). Briefly, 4 g of $NbCl_5$ was added to a mixture of 25 mL of water and 4 mL of H_2O_2 (30 % w/w), under agitation for 10 min. We then added surfactants (0.1 or 0.5 mmol) during mixing, resulting in the samples described in **Table 1.** The suspension was then added to a microwave setup inside a reactor cell at 75 ˚C for 3 h under controlled MW radiation. Following synthesis, the precipitate was isolated via centrifugation at 5000 rpm for 10 minutes and subjected to five successive washing cycles with deionized water to ensure complete removal of residual impurities. This standardized purification procedure was rigorously applied to all samples to maintain experimental consistency. The compounds were then dried in a conventional oven at 75 ˚C for 24 h and maintained in the dark at room temperature until further use.

Table 1: Niobium oxide samples prepared through the Microwave Method

Sample abbreviation	**Synthesis composition**
NbSDS5	SDS 0.5 mmol/Nb_2O_5
NbSDS1	SDS 0.1 mmol/Nb_2O_5
NbCTAB5	CTAB 0.5 mmol/Nb_2O_5
NbCTAB1	CTAB 0.1 mmol/Nb_2O_5
NbCA5	CA 0.5 mmol/Nb_2O_5
NbCA1	CA 0.1 mmol/Nb_2O_5
NbOA1	OA 0.5 mmol/Nb_2O_5
NbOA5	OA 0.1 mmol/Nb_2O_5
Nb_2O_5	Nb_2O_5

CA - citric acid; OA – Oleic acid; SDS – Sodium dodecyl sulphate; CA – Citric acid; CTAB – Hexadecyltrimethylammonium bromide.

2.3 Evaluation of the acidity by TPD-NH_3

The evaluation of acidic sites for each catalyst was performed using the Temperature-Programmed Desorption technique with ammonia as a probe molecule (TPD-NH_3). We weighed the samples at approximately 100 mg and added them to a quartz reactor between two balls of quartz wool. Two stainless steel cylinders protected the reactor. Each sample was raised at 160 °C for 1 hour in a He flow (30 mL/min) prior to the adsorption of ammonia. The sample was then saturated with NH_3 for 30 min at 100 °C. He (30 mL/min) was used to desorb the physically adsorbed ammonia from the sample for 60 min at 100 °C. The temperature was raised from 100 °C to 600 °C at a rate of 5 °C/min to evaluate by desorption the chemically adsorbed ammonia using a thermal conductivity detector. Deconvolution graphs were performed using Fityk (Wojdyr, 2010) and Origin Pro Learning Edition Version 2024 (Sydney, Australia).

2.4 Determination of Surface area by N_2 physisorption

Samples (~200 mg) were evaluated regarding their surface area using the Micromeritics® TriStar II Plus (Georgia, USA). Each sample was previously degassed with vacuum and He (90 ˚C for 30 min, 250 ˚C for 120 min), and the surface area was measured based on nitrogen physisorption at 77 K (-196 ˚C). Calculations were executed in the built-in software by Brauner-Emmet-Teller (BET) for surface area and Barrett-Joyner-Halenda (BJH) for pore distribution.

2.5 Characterization of Chemical structure by Raman, FT-IR and XPS spectroscopies

Raman spectroscopy was used to acquire accurate details on the chemical structure of surface-modified Nb_2O_5. For that, the results were collected using a Voyage, BWS. 435-785HY (Madatec, Florida, USA). The excitation wavelength was 785 nm, and the analyses were performed at room temperature.

The possible addition of chemical groups from the surfactants was evaluated by Fourier-transform infrared spectroscopy (FT-IR) with a Shimadzu IR-Spirit (Kyoto, Japan). Samples were analysed in the attenuated total reflectance (45 scans) model MIRacle (Pike Technologies, USA). The reading was adjusted between 4000–400 cm^{-1}. Samples were dried at 150 ºC for 12 h prior to the analysis to remove any humidity and cooled to room temperature inside a desiccator.

Spectra were performed with Origin Pro Learning Edition Version 2024 (Sydney, Australia).

2.6 Characterization of Surface morphology by SEM

Surface morphology was tested by scanning electron microscopy (SEM) using JSM 6610LV, (JEOL, Japan). The excitation voltage was set to 15 kV.

2.7 Determination of Crystalline structure by XRD

The crystalline structures of each Nb_2O_5 sample were analysed using X-Ray Diffraction (XRD) with a Shimadzu 6000 equipment (Japan). The 2θ reflection angle was monitored from 10 to 80° at a scanning rate of 2° min^{-1} and 0.02º step, at 30 kV, 30 mA, and CuKα radiation (λ = 1.5418 Å). Origin Pro Learning Edition Version 2024 was used as statistical software.

2.8 Cytotoxicity assays

2.8.1 Hemolytic and anticoagulant activities

To evaluate the erythrocyte membrane rupture from each compound, a hemolytic activity was performed according to our previous study (Cerveira *et al.*, 2021). Briefly, 1 mg, 5 mg, and 10 mg of each Nb_2O_5 sample were added to a 4 % erythrocyte solution from defibrinated sheep's blood in PBS. The suspension was then left to react at 37 °C for 1 h under agitation (150 rpm). The supernatant was separated through centrifugation for 10 minutes at 800 g. PBS was used as a negative control (NC) and Triton-X100 as the positive control (PC). The percentage of membrane rupture was calculated as (AT x AC/AP - AC) x 100, where AT is the absorbance of the treated supernatant and AP and AC are the absorbances of the positive and negative controls, respectively. The absorbance was read in a Polaris microplate reader (Celer, Belo Horizonte, Brazil). A two-way ANOVA followed by Dunnett's *post hoc* was performed to analyze the data using GraphPad Prism 10 (California, USA).

To evaluate the anticoagulant behavior, we tested whether the test compounds might alter the clotting time of activated partial thromboplastin time (aPTT) and the prothrombin time (PT). The citrated plasma was in contact with the different concentrations (1, 5 and 10 mg/ml) for a 30-min period at 37 °C, with shaking (55 rpm). Subsequently, the tubes were centrifuged for 10 min at 2000 rpm, and the supernatant was used for the analysis. aPPT and PT were determined using commercial kits (Biotecnica, Brazil), following the manufacturer's instructions. Samples were read in two independent duplicates using a bichannel coagulometer (COAG-1000, Wama Diagnostics, Switzerland).

2.8.2 Cell viability

The ZF-L cell line was purchased from the Rio de Janeiro Cell Bank (Rio de Janeiro, Brazil). Cells were maintained until use in 50 % L-15, 35 % DMEM high glucose, and 15 % Ham's F-12. The medium was supplemented with 0.15 g/L $NaHCO_3$, 15 mM 4-(2-Hydroxyethyl) piperazine-1-ethane-sulfonic acid (HEPES), 0.01 mg/mL insulin, 10% (v/v) FBS, 50 µg/mL enrofloxacin, and 2.5 µg/mL amphotericin B. The cells were kept in a dry oven at 28 °C until reaching 90 % of confluence.

ZF-L cells were seeded in 96-well plates at a density of $1x10^5$ cells/well. Prior to the treatment, samples were weighted at 1 mg, 5 mg, and 10 mg and added to the complete medium for 24 h at 28 °C. The samples were centrifuged for 5 min, and 100 µL of the supernatant was added to the cells and incubated for 24 h at 28 °C. After the incubation period, mitochondrial viability was determined according to the method by Mosmann (1983) (Mosmann, 1983), with modifications (Ferrer *et al.*, 2024). The MTT is based on the ability of live mitochondria to reduce the (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) from yellow to blue formazan crystals. The MTT solution (1 mg/mL in complete medium without FBS, pH 7.4) was added and incubated in an oven at 28 °C for 3 h. The medium was removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The plate was read at 496 and 630 nm using a Polaris microplate reader (Celer, Belo Horizonte, Brazil). The percentage of cell viability was calculated using the formula AT/AC × 100, where AT is the absorbance of the treated cells and AC is the negative control (NC). DMSO was used as a positive control (PC). A two-way ANOVA followed by Dunnett's *post hoc* was performed to analyse the data using GraphPad Prism 10 (California, USA).

2.9 Nb composition and leaching

To evaluate Nb composition on freshly synthesized samples and their leaching to the medium, Energy-dispersive X-ray spectroscopy (EDX) was performed. Solid samples did not require any preparation prior to the analysis. For Nb leaching, 1 mg, 5 mg and 10 mg were added to complete ZF-L medium without FBS for 24 h at 28 °C. The EDX-720 (Shimadzu, Kyoto, Japan) was set to a 50 kV voltage, a Rhodium tube, a 5.0 mm collimated beam, and a liquid N_2-cooling detector. Samples were placed in a 25 mm x 5 µm PVC material chamber, and a random liquid portion was evaluated for the presence of Nb. The test was performed in triplicates, and the amount obtained was corrected with the diameter of the recipient's test. Data is shown as mean ± SD.

3. Results and discussion

3.1 Acid sites

The total and relative acid sites resulting from Nb_2O_5 modified samples are shown in **Table 2**. Data was obtained from deconvolution graphs. The relative desorption is related to the normalized peak area with respect to the total desorbed ammonia.

Results indicate that most samples display very weak (100-260 °C) and weak (260-360 °C) acid sites, with more than 50% of their acidity relying on this temperature range, probably linked to Lewis's acid sites. NbSDS5 showed more than 70 % of its acidic behaviour in the weak acidity range, while NbCTAB1 had less than 15 %. The results suggest that the materials primarily interact with the ammonia molecules in a weak-moderate bound state, primarily through medium-strength chemisorption. NbSDS1 and NbSDS5 probably have more acid sites as they have fewer deconvolution peaks and bigger peak widths.

The commercially available Nb_2O_5 was evaluated using TPD-NH_3, and the total desorbed ammonia was only 18 µmol/g (Eblagon *et al.*, 2020). The amorphous Nb_2O_5 was found to have 859 µmol/g (de Lima *et al.*, 2021), with mostly sites in the weak acidity temperatures. Our samples were much more acidic, with pure Nb_2O_5 reaching 2015 µmol/g and NbSDS5 at 3140.7 µmol/g, but they do corroborate with many peaks in the weaker zone. The synthesis methods and settings may have influenced these differences in the final product.

Although the TPD-NH_3 technique is normally used for catalyst characterization, it could be beneficial for a biomedical application to understand how the charges behave on the material's surface. Brønsted and Lewis acid sites can potentially interact with different biomolecules, such as proteins and lipids. Silver nanoparticles, for example, can bond to enzymes and DNA, altering metabolism and DNA replication (Lemire; Harrison; Turner, 2013; Morones *et al.*, 2005). Mitta *et al.* (2017) showed that Nb^{5+} can interact with salmon DNA and alter the chemical stability and structural properties (Mitta *et al.*, 2017).

Millan, Susrisweta, and Sahoo (2023) demonstrated the interaction of Nb_2O_5 with bovine serum albumin (BSA) and human serum albumin (HSA). The oxide caused changes in fluorescence quenching and alterations in the secondary and tertiary conformations. Phosphopeptides can be enriched in the presence of Nb_2O_5 from the digest of a-casein, with a recovery of almost 100% for the peptide sequence VNQIGpTLSESIK (Ficarro *et al.*, 2008). Schardosim et al. (2021) DNA damage and chromosome impairment when cells were exposed to Nb_2O_5.

Adding a Brønsted acid site to a Lewis catalyst raised the conversion rate of lactose to 78 % when the catalyst and substrate were mixed in a 1:1 ratio (Yi *et al.*, 2024). Although this research is focused on the industrial application, it can also demonstrate the interactions of acid sites with enzyme activity, as it enables a better cleavage of the β-1,4 glycosidic bond in lactose.

Human carbonic anhydrase II requires Zn as a cofactor, and it its coordinated to the protein by three imidazole groups from histidine residues. The Zn ion acts as a Lewis acid in the protein by lowering the pKa of the Zn-H_2O bond to form a Zn-OH^- complex. This results in a proton transfer to a histidine residue, ensuring the reactivity of the site. Consequently, it allows the rapid conversion of CO_2 into HCO_3^- and thus maintains blood homeostasis (Carlsson; Jonsson, 2000; Kyun Kim *et al.*, 2018).

3.2 Surface area

The surface area, pore volume, and pore diameter are resumed in **Table 3**. Nb_2O_5 sample appeared as microporous (average pore diameter < 5 nm), but after the addition of surfactants, all samples were classified as mesoporous and displayed a decrease in the specific area significant according to the International Union of Pure and Applied Chemistry (IUPAC) classification. With the increase of surfactant quantity in the synthesis, the surface area remained in the same order of magnitude for the samples NbCA and NbOA but underwent a significant decrease for NbSDS (from 164.0 to 36.3 m^2/g) and NbCTAB (from 212.0 to 114.9 m^2/g).

Moreover, for all samples, the pore distribution profile remained the same but with an increase in the average size, and the total pore volume was slightly reduced. The exception was for NbOA material, which maintained practically the same value for total pore volume.

Table 2: Acidity of the catalysts evaluated by Total and Relative NH_3 Desorption (µmol/g)

SAMPLE NAME	Very Weak Acidity (100-260 °C)	Weak Acidity (260-360 °C)	Strong Acidity (360-445 °C)	Very Strong Acidity (> 445 °C)	Total
Nb_2O_5	1054 (52%)	805 (40%)	45 (2%)	111 (6%)	2015
Anionic surfactants					
NbOA1	642.23 (30.99%)	795.07 (38.36%)	367.47 (17.73%)	267.76 (12.92%)	2072.54
NbOA5	563.35 (32.10%)	898.54 (51.20%)	109.25 (6.22%)	183.96 (10.48%)	1755.1
NbCA1	952.76 (45.81%)	857.12 (41.21%)	-	270.15 (12.99%)	2080.03
NbCA5	127.07 (4.61%)	1906.8 (69.16%)	506.74 (18.38%)	216.42 (7.85%)	2752.03
Cationic surfactants					
NbCTAB1	1646.75 (68.59%)	351.84 (14.65%)	57.11 (2.38%)	345.26 (14.38%)	2400.96
NbCTAB5	848.01 (44.20%)	648.93 (33.82%)	358.95 (18.71%)	62.60 (3.26%)	1918.48
NbSDS1	1098.06 (49.82%)	733.09 (33.26%)	-	373 (16.92%)	2204.15
NbSDS5	316.44 (10.08%)	2233.65 (71.13%)	333.64 (10.63%)	256.34 (8.16%)	3140.7

Our study found that amorphous Nb_2O_5 obtained from the microwave technique had a surface area of 230.4 m^2/g. Eblagon *et al.* (2020) had a surface area of 366 m^2/g for the amorphous sol-gel Nb_2O_5 dried at 100 °C, and for the commercially Nb_2O_5 used for comparison, they found 4 m^2/g (Eblagon *et al.*, 2020). Lima *et al* (2021) obtained a surface area of 142 m^2/g for an amorphous Nb_2O_5 synthesized from the hydrothermal method, with a pore volume of 0.16 cm^3/g and an average pore diameter of 44 Å (de Lima *et al.*, 2021). Moreover, Li *et al* (2015) obtained a surface area for amorphous Nb_2O_5 of 129.6 m^2/g from a sol-gel synthesis (Li, Shuang *et al.*, 2016).

3.3 Chemical structure

One of the major problems with the use of metal oxide nanoparticles (NP) is their tendency to form agglomerates due to their characteristics (Shah *et al.*, 2017), such as high surface area and acidic sites. Consequently, the modification of these particles appears as an alternative to try to solve or improve this problem. Surface modifications with surfactants can be accomplished either by chemical or physical adsorption, depending on the functional groups present. Chemical adsorption occurs mainly in the presence of thiol groups (Heinz *et al.*, 2017), establishing covalent bonds between the substances, resulting in proton release. In physical adsorption, the formation of electrostatic interactions is established, often with the hydrophilic part, increasing the NP's affinity for organic molecules (Choi *et al.*, 2020).

The application of surfactants to regulate the size distribution of NPs is essential in areas such as sensors, as it is necessary to identify very small traces of material (Shaban; Kang; Kim, 2020). Quercetin nanomicelles with the surfactant Pluronic-F127 were evaluated for the detection of Cu^{2+} ions and cysteine residues, which may be linked to neurological conditions (Jiang *et al.*, 2018). The synthesis of Nb_2O_5 nanorods with the addition of different surfactants demonstrated the influence on morphology, dispersion, and crystallinity (Ali; Nazemi; Gates, 2017)

The curve analysis focused on the 100–800 cm^{-1} range of the spectrum, as this range offers substantial evidence of Raman scattering for adjacent atoms in the analyzed sets. All samples displayed faint active modes at frequencies of 74, 233, 485, and 664 cm^{-1}, with increased intensities observed at 74 and 664 cm^{-1}, suggesting the presence of lower symmetry phases in the Nb_2O_5 (**Figure 1a).** These manifestations are related to the vibrational modes of the NbO_6 octahedra (Riemke *et al.*, 2022; Ücker *et al.*, 2019) behaviour characteristic of amorphous and low crystallinity samples.

Figure 1b shows the FTIR spectra of Nb_2O_5-based samples. The band at 1620 cm^{-1} can be attributed to angular deformation of the Nb-OH bond (Cardoso *et al.*, 2012) but also bending vibrations from water molecules (Crisóstomo *et al.*, 2024). The signal at 600 cm^{-1} can be associated with Nb-O, and the stretching around 800 cm^{-1} corresponds to asymmetric stretching of O-Nb-O bonds (Burcham; Datka; Wachs, 1999). Furthermore, the small peak around 900 cm^{-1} is related to the Nb=O bond (Brum *et al.*, 2025). All these stretching vibrations are related to the presence of Nb_2O_5 in all samples, although we did not see any significant modifications or presence of functional groups with the addition of different surfactants during the *in-situ* synthesis.

Table 3: Surface area (BET) and Pore Size (BJH) analysis for Nb_2O_5 and modified Nb_2O_5 samples

Samples MW	Surface area (m^2/g)	Average pore diameter (nm)	Total pore volume (cm^3/g)
Nb_2O_5	230.4	2.0	1.7
NbCA1	161.3	6.5	0.04
NbCA5	167.9	9.4	0.01
NbOA1	89.5	20.0	0.01
NbOA5	93.9	26.4	0.01
NbSDS1	164.0	8.1	0.03
NbSDS5	36.3	10.1	0.02
NbCTAB1	212.0	7.4	0.04
NbCTAB5	114.9	22.0	0.03

3.4 Surface morphology

Surfactants are added to the synthesis process to potentially reduce nanoparticle aggregation (Ali; Nazemi; Gates, 2017). This may stem from their amphiphilic nature since the polar head group causes a repulsion with the surface. SEM images were taken **(Figure 2)** to analyze morphological changes in the materials. In our study, the particles seem to adopt a spherical shape. However, all samples present a tendency to form aggregates of nanoparticles, similar to a bunch of grapes. This indicates that the incorporation of surfactants during synthesis did not facilitate their dispersion. The small nanoparticles appear to have a tendency to form clusters that could result from the acidic environment and the influence of Van der Waals forces (Bélteky *et al.*, 2021; Endres; Ciacchi; Mädler, 2021; Shrestha; Wang; Dutta, 2020)

Functional groups in surfactants, such as carboxylate, sulfonate, and sulfate, were prone to reducing aggregation during the synthesis of Nb_2O_5 nanorods by the hydrothermal method. This effect could be attributed not only to their amphiphilic nature but also to the different synthesis process. The addition of an N-containing surfactant showed more clustered and less uniform nanorods (Ali; Nazemi; Gates, 2017). Moreover, Vosoughifar (2016) showed that the addition of SDS can decrease the nanoparticle size better than EDTA in a Nb_2O_5 sample calcined at 700 °C (Vosoughifar, 2017). In this study, the addition of surfactants in the synthesis process did not seem to cause any changes in the Nb_2O_5 morphology, but they seem to alter the surface area and the acid sites as previously discussed. The clusters do not have an equal average size, although the equipment used had a limited resolution to evaluate the particles.

3.5 Crystallinity

Figure 3 illustrates the X-ray diffraction (XRD) patterns of pure Nb_2O_5 and Nb_2O_5 synthesized with different surfactants. The X-ray diffraction profiles exhibit a broad spectrum across a large range of 2θ angles, confirming the presence of dispersed reflections from nanocrystalline materials. The observed wide-band diffractions in all specimens align with the properties of nanocrystalline Nb_2O_5 formations (Riemke *et al.*, 2022). No notable changes were observed among the specimens synthesized, indicating that the surfactant concentrations used were insufficient to force the NbO_6 octahedral building blocks of Nb_2O_5 into long-range arrays throughout the creation of any Nb_2O_5 polymorphs.

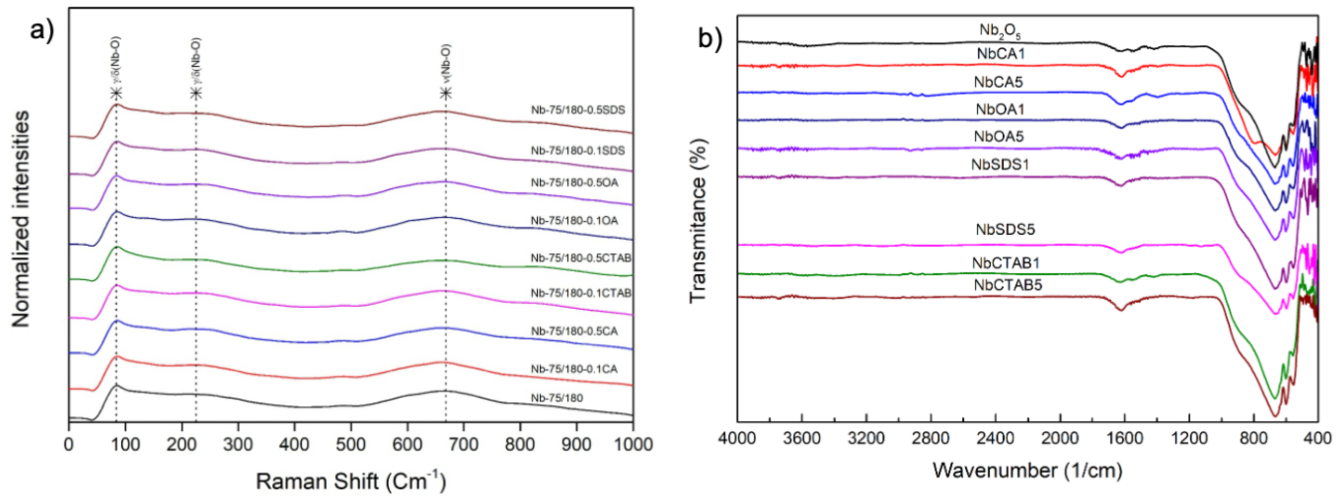

Figure. 1. (a) Raman and (b) Fourier transform infrared (FTIR) spectra for each sample.

2.6 Cytotoxicity

Erythrocytes are widely used as a cell model for several reasons, including (1) ease of reading as the release of hemoglobin can be measured spectrophotometrically; (2) simplified model as they are anucleated; and (3) a large abundance in the human body (Pagano; Faggio, 2015). The study of red blood cell (RBC) toxicity is of great importance as they are responsible for oxygen transportation. In this study, we evaluated different surfactant-modified Nb_2O_5 samples. As demonstrated in **Figure 4a**, all samples showed a concentration-dependent activity. All materials were safe at 1 mg/mL, but some showed cytotoxic activity at 5 and 10 mg/mL. At 10 mg/mL, Nb_2O_5 alone showed significant cytotoxicity, resulting in less than 15 % of erythrocyte viability. The safest compound was NbCTAB5, with only 26 % of RBC lost at 10 mg/mL. The concentrations needed to kill 50 % of erythrocytes (IC_{50}) are shown in **Table 4**. The lowest IC_{50} was obtained for NbCA1, and the highest for NbCTAB5. Some of the values were extrapolated by the software.

It is unlikely that the Nb leaching caused any competition with Fe for the heme complex, given the neutral blood pH. However, the acidic surface of the samples may interact with the RBC and/or its proteins in the lipid bilayer. This could weaken the membrane and thus cause hemolysis. Moreover, their acidic surface can promote sites for reactive oxygen species (ROS) formation. MNPs can alter the cell morphology (Kozelskaya *et al.*, 2016) and rigidity (Pan *et al.*, 2018). Modulation of surface properties with surfactants can also interfere with the interaction with RBC (Peetla; Labhasetwar, 2009)

TiO_2 nanoparticles, for example, can show signs of spherocytosis and echinocytosis when exposed to human RBC (Ghosh; Chakraborty; Mukherjee, 2013), and changes in white blood cell count. Unmodified silver nanoparticles (AgNP) do not show any hemolytic activity, not even after 24 h (Korolev *et al.*, 2021). ZnO NP were found to cause hemolysis through oxidative stress, lipid peroxidation, and glutathione depletion in a concentration-dependent behavior (0.3 to 1 mM) (Salami; Khosravi; Zarei, 2022).

Unfortunately, we did not have the resources at the time to further evaluate the interaction of the samples with RBC components. It is known, however, that Nb interacts with HSA by causing changes in the secondary and tertiary structures and in the protein's polarity by modifications in the fluorophore residues (Millan; Susrisweta; Sahoo, 2023).

To the best of our knowledge, there is no other research available in the literature about the hemolytic activity of Nb_2O_5 nanoparticles. Dsouki et al. (2014) evaluated the blood alterations of a 3 % commercial Nb_2O_5 solution (1 mL) injected intraperitoneally in Swiss male mice. No changes were observed during the 12^{th}-day treatment for hematological parameters.

The clotting time is also another important parameter to consider when testing a new compound's toxicity. This is a great indicator in vitro to evaluate the tendency of a NP to induce any platelet aggregation.

Our studies revealed that most samples maintained the PT (**Figure 4b**) levels close to the control time, indicating no interference with the extrinsic pathway. The exception was for Nb_2O_5 at 5 and 10 mg/ml, where the average time was 5.75 ± 0.25 and 5.9 ± 1.9, respectively, and for NbCA1 (4.4 ± 0.1). Although these values were not considered as significant during the statistical analyses, there was a high decrease in clotting time. Moreover, NbOA10 did not cause any clotting during the experiment time (2 minutes).

The intrinsic pathway was altered for a great number of samples through the TTPa (**Figure 4c**). NbCTAB1 (1 mg/ml), NbCA1 (5 mg/ml), and Nb_2O_5, NbOA5, NbSDS5, NbCTAB5 (10 mg/ml) did not coagulate within the 2-min time. Further studies must be performed to evaluate which factor from the intrinsic pathway (factors IX, XII or XII) was the most affected. Moreover, the fibrinogen and the Ca^{+2} uptake could also be affected.

Chitosan-functionalized silver nanoparticles, for example, can increase both aPTT and PT in white male albino rabbits (Asghar *et al.*, 2020; Lozano-Fernández *et al.*, 2019). The evaluation of ZnO (I and II) and TiO_2 NP also showed an increased and decreased, respectively, in PT (Lozano-Fernández), and a significantly increased TTPa for all samples. ZnO (II) at the concentration of 1 mg/ml did not result in any intrinsic coagulation during the experiment time.

It is known that the ZnO interferes with the conformational arrangement of human fibrinogen (Simón-Vázquez *et al.*, 2014), causing a weaker clot. TiO_2 nanocrystals showed the same behavior, while nanotubes enhanced the strength of the clot (Millan; Susrisweta; Sahoo, 2023; Roy; Paulose; Grimes, 2007). NP can also adsorb coagulation factors on their surface (Yang *et al.*, 2017) and the formation of protein coronas (Nishihira *et al.*, 2019) which could reduce the availability in the plasma and hence delay the coagulation.

Moreover, we did not find in the literature any studies on the Nb_2O_5 interference in the coagulation cascade. However, it was already discovered that Nb_2O_5 can interact with albumin (Millan; Susrisweta; Sahoo, 2023), the most abundant protein in plasma, causing changes in the secondary and tertiary structures. In that sense, low levels of functionalized albumin in the blood can contribute to thromboembolism, whilst higher levels can prevent clot formation (Paar *et al.*, 2017) As our study revealed that Nb ions can dissociate to the oxide, it could also compete with Ca^{2+} ions in the coagulation cascade, as Nb can be found in the +2-oxidation state.

To evaluate the cytotoxicity of the modified Nb_2O_5, they were exposed to the ZF-L cell line (**Figure 5**). Prior to the treatment, the extracts (complete medium with 1 mg, 5 mg, or 10 mg of each material) were prepared with constant agitation for 24 h. Due to their acidic nature, the pH shift occurred either immediately or after the incubation time. It was not possible to measure the pH due to the small volume of each extract, but it is known that phenol red (used as a pH indicator) changes to its zwitterion form under pH 6,

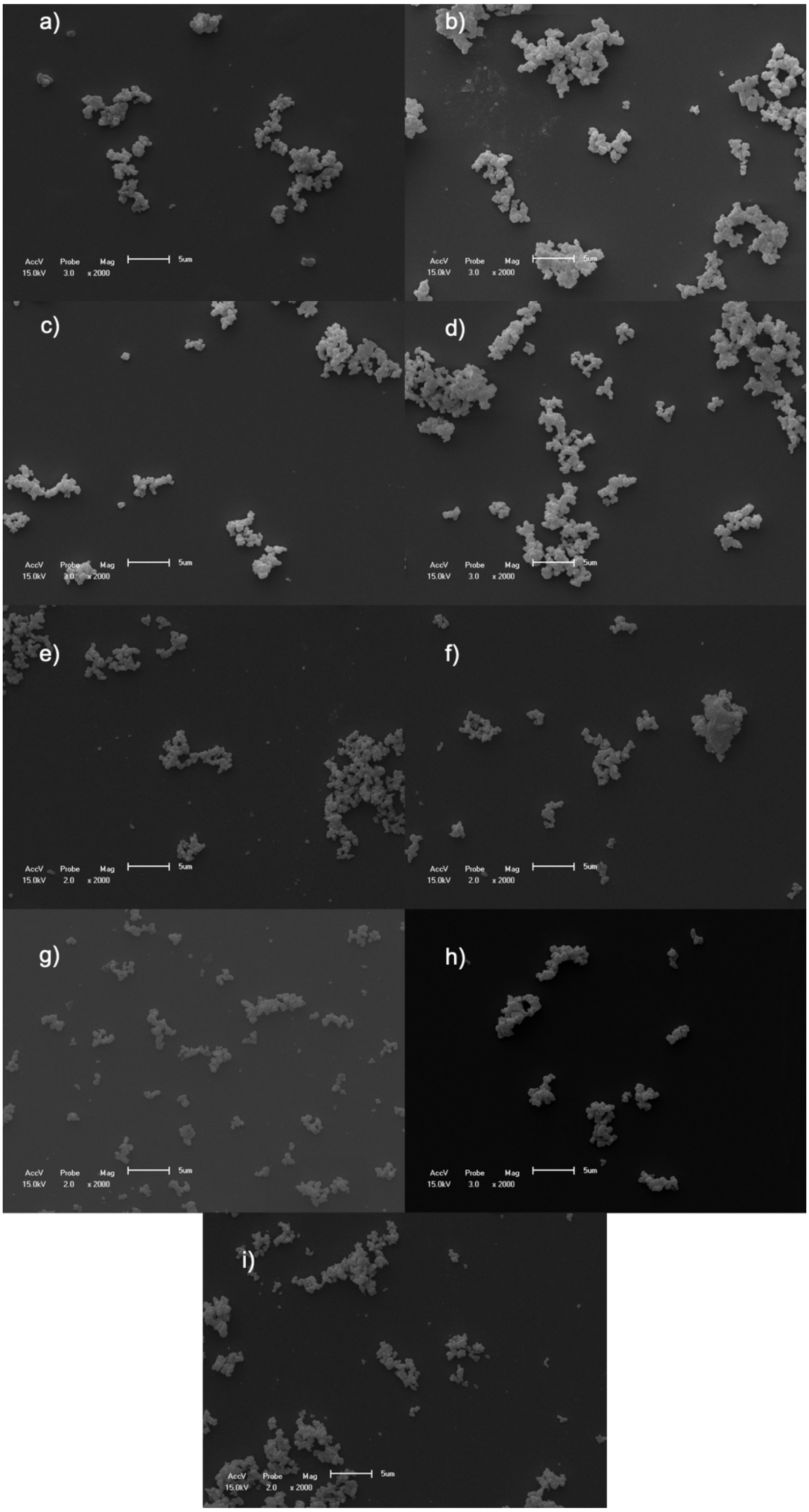

Figure 2. Scanning electron microscopy (SEM) images of the samples (a) NbCA1, (b) NbCA5, (c) NbOA1, (d) NbOA5, (e) NbSDS1, (f) NbSDS5, (g) NbCTAB1, (h) NbCTAB5, and (i) Nb_2O_5.

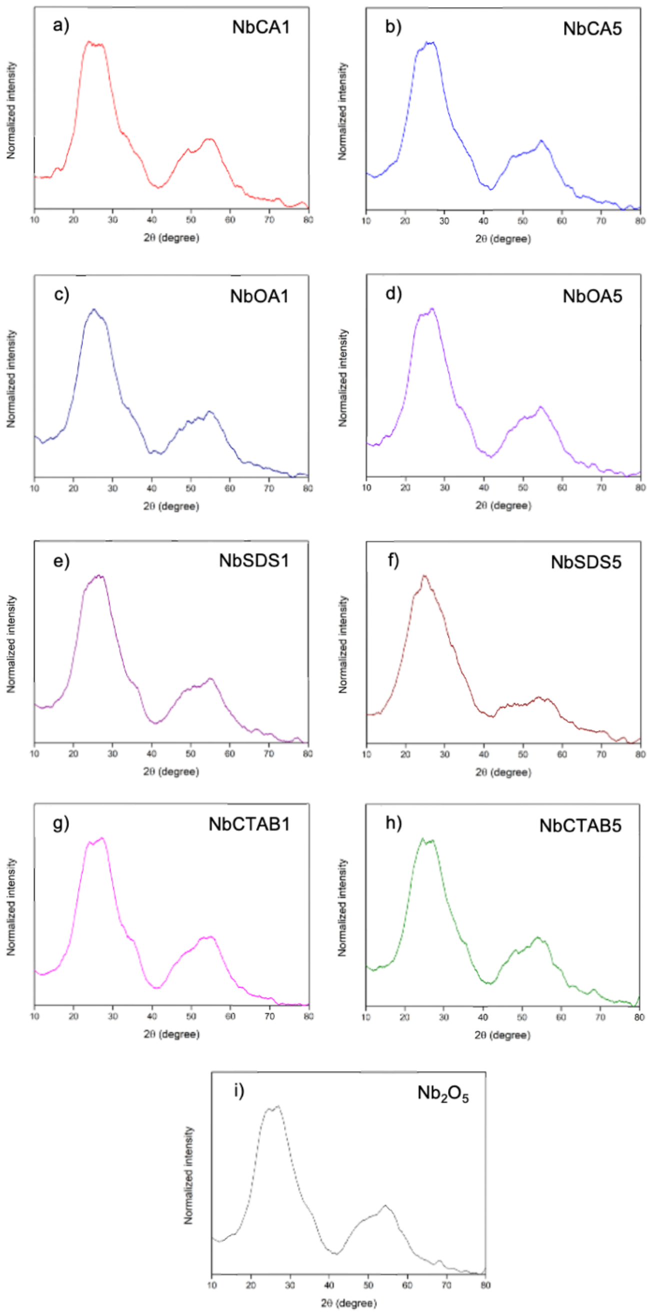

Figure 3. X-Ray Diffraction (XRD) patterns for (a) NbCA1, (b) NbCA5, (c) NbOA1, (d) NbOA5, (e) NbSDS1, (f) NbSDS5, (g) NbCTAB1, (h) NbCTAB5, and (i) Nb_2O_5.

displaying a yellowish color (Weiskirchen *et al.*, 2023). A chronic exposure (> 7 days) experiment should be performed to evaluate the long-term adaptation of ZF-L in an acidic environment, as a decrease in pH by more than 1.5 points cannot be considered acceptable (Fenner, [*s. d.*]). Zahangir et al. (2015) found that the average acid pH to cause 50 % of mortality is 3.9 in adult zebrafish.

Interestingly, the ZF-L was able to resist the pH alteration. According to the Cell Bank of Rio de Janeiro, which supplied the cells, the ideal pH range is between

7.0 and 7.6. The materials only caused an increased cell death at 10 mg/mL, especially NbOA5 (13.25 % cell viability) and NbCA1 (27.12 % cell viability) Pure Nb_2O_5 had a 94.88 % of cell viability at the lowest concentration (1 mg/mL) and 46.99 % at 10 mg/mL. At 5 mg/mL, it enhanced cell survival by 1-13-fold. NbSDS1, at 1 mg/mL, increased cell viability by 1-3-fold, even at lower and non-physiological pH conditions **(Figure 5)**.

The extract of amorphous Nb_2O_5 in distilled water (concentration of 1 m^2 BET surface area) was evaluated in L929 mouse fibroblast cell viability, but no significant changes were observed even after 30 days (Pradhan *et al.*, 2016). However, in CHO-K1 cells, amorphous Nb_2O_5 showed DNA damage at 210 µg/mL (Schardosim *et al.*, 2022).

The monoclinic pure Nb_2O_5 demonstrated to reduce by 60 % the inhibition of L20B and by 33.9 % of MC7 cells (Salim *et al.*, 2022). When used as a coating for the SS 316L, Nb_2O_5 showed a reduction in the TNF-α and IL-17 levels in HGF-1 cells (Moreto *et al.*, 2021). It also had an increase in IL-10 for VERO-CCL-81 cells, and a reduction in apoptosis levels, helper T cells, cytolytic T cells, and PMN cells when coating Ti6Al4V (de Almeida Bino *et al.*, 2021a).

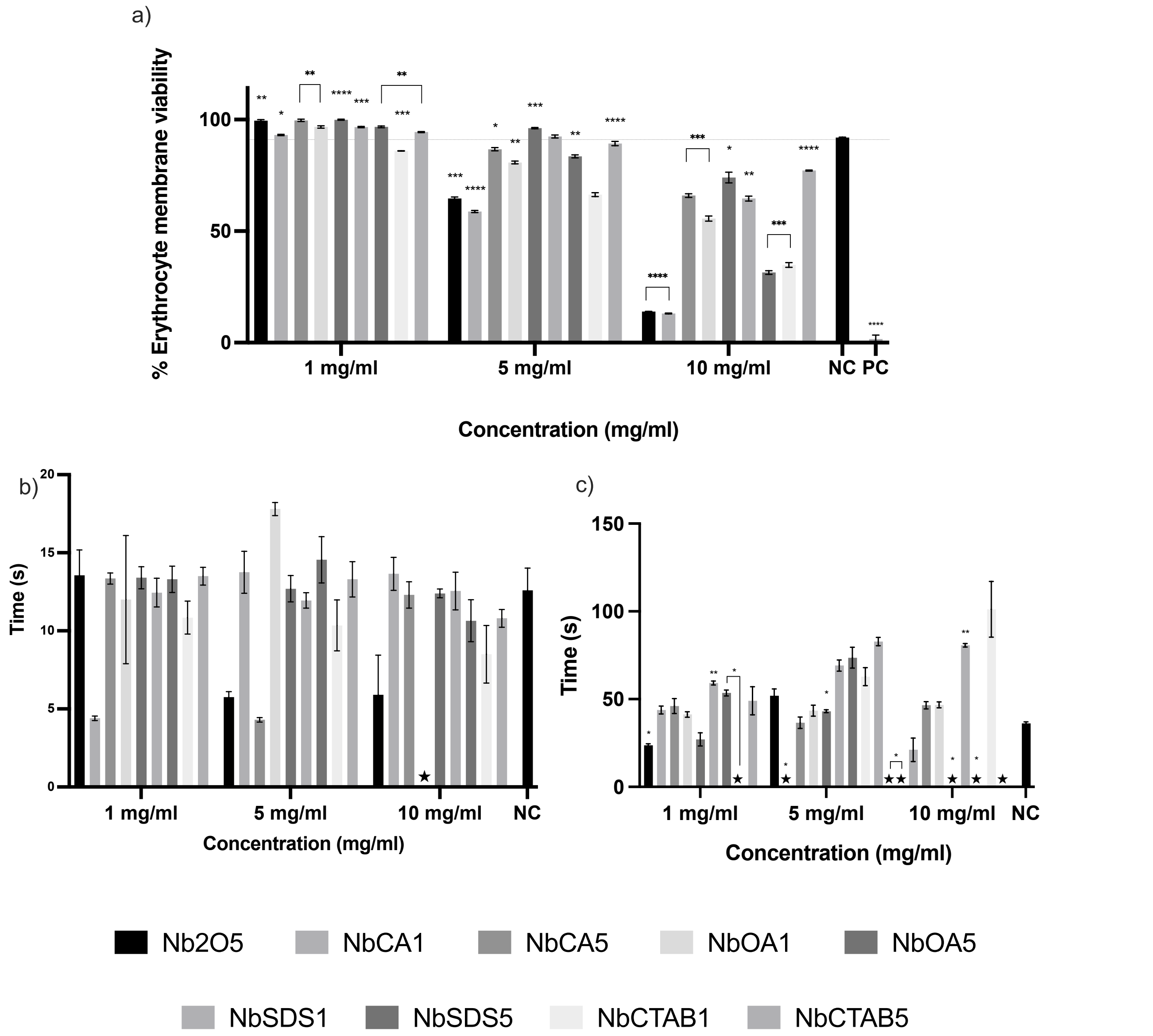

Figure 4. Evaluation of a) hemolytic activity, b) PT, and c) TTPa of different modified Nb_2O_5 Star means that no coagulation was observed within 120 s. Data are expressed as mean ± SD from triplicate readings. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

According to the EDX analysis **(Table 5),** samples had over 98% of Nb content in their composition. While this equipment cannot assess the oxygen concentration, we may conclude, in conjunction with FTIR, RAMAN, and DRX spectra, that no additional components are present in any of the structures. Moreover, all samples exhibited Nb leaching into the medium. The increase in Nb_2O_5 concentration did result in a proportional increase in Nb leaching, except for NbCA1. Further studies are required to understand how Nb ions are released into the medium and how they interact with cell metabolism. The highest Nb leaching was observed for NbOA1 10 mg/mL (4.93 mg/mL), followed by NbSDS 10 mg/mL (4.22 mg/mL) and NbOA1 5 mg/mL (3.39 mg/mL).

NbSDS5, NbOA5, and Nb_2O_5 had a considerable decrease in cell survival at 10 mg/ml. Moreover, Nb leaching did not seem to have a direct relationship with cell viability, cell proliferation, or hemolysis, although the exact activity by which Nb ions interact in cell pathways remains unclear. It is known, however, that Nb can localize in lysosomes (Berry; Bertrand; Galle, 1993) possibly due to its interaction with acid phosphatase, which hydrolyzes phosphate groups under acidic conditions (Anand; Srivastava, 2012).

We also used Pearson's correlation to assess how the acid sites and surface area related to the hemolytic activity, anticoagulant, and cytotoxicity assay, as shown in **Table 6**.

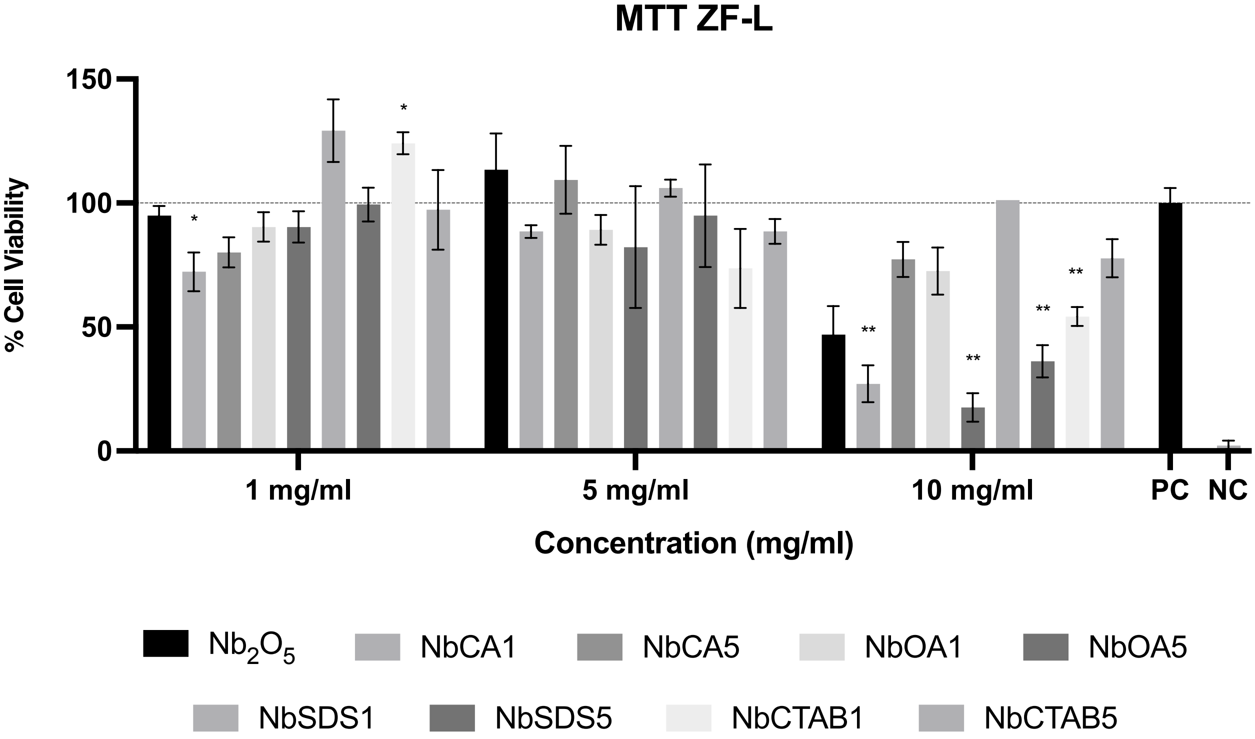

Figure 5. Cytotoxicity activity in ZF-L cell line. Data is expresses as mean ± SD from triplicate readings. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 4: IC_{50} values for erythrocyte membrane rupture (mg/mL)

	Nb_2O_5	**NbCA1**	**NbCA5**	**NbOA1**	**NbOA5**	**NbSDS1**	**NbSDS5**	**NbCTAB1**	**NbCTAB5**
HA*	5.942	5.567	14.48	11.49	13.95	12.5	7.984	7.001	38.68

*HA: Hemolytic activity; %ZF-L: Cytotoxicity in ZF-L cell line.

There was no relevant alteration in surface area for the samples. The hemolytic activity for 1 mg/mL was significant (r = -0.8853, p = 0.0015), indicating an inversely proportional relation (as the r is negative). MTT had the same behavior at 5 mg/ml (r = -0.7275, p = 0.0263), while TTPa at 1 mg/ml found a significance proportionally related (r = 0.7053, p = 0.0338). We performed a correlation between MTT and EDX analysis using the same solution. No significance was found (data not shown).

In mesoporous silica nanoparticles, there is a direct correlation between the surface area and the cytotoxicity in HeLa cells, increasing ROS(She *et al.*, 2020). Another study showed that the cytotoxicity decreased as the pore volume increased in J77A macrophages, but in EAHY926 and 3T3 cells, the smaller the diameter, the higher the cytotoxicity (Rabolli *et al.*, 2010). More studies are necessary to understand the exact influence on the superficial changes of Nb_2O_5 in the cytotoxicity assays.

Table 5: Nb content of fresh samples (%) and Nb leaching into complete medium (mg/mL)

	NbCA1	**NbCA5**	**NbOA1**	**NbOA5**	**NbSDS1**	**NbSDS5**	**NbCTAB1**	**NbCTAB5**	**Nb_2O_5**
F.S.*	98.5% ± 2.6	100 %	100 %	100 %	100 %	100 %	99.9 % ± 0.17	100%	99.9 ± 0.01
1 mg/ml	3.02 ± 0.75	0.3 ± 0.1	0.14 ± 0.01	XX	0.64 ± 0.38	0.62 ± 0.07	0.25 ± 0.14	0.25 ± 0.14	0.73 ± 0.12
5 mg/ml	2.14 ± 1.33	0.94 ± 0.1	3.39 ± 0.44	0.83 ± 0.08	0.76 ± 0.16	1.77 ± 0.28	0.73 ± 0.09	1.48 ± 0.02	1.69 ± 0.31
10 mg/ml	1.84 ± 0.05	2.6 ± 0.22	4.93 ± 1.22	XX	3.62 ± 0.18	4.22 ± 1.84	3.32 ± 0.003	2.47 ± 1.34	3.02 ± 0.75

*F.S: Freshly synthesized samples.

Table 6: Pearson's correlation for Nb_2O_5 modified sample.

		S.A* Pearson r	**p value**	**A.S% Pearson r**	**p value**
Hemolysis	1 mg/mL	-0.4849	0.1811	-0.8853	0.0015**
	5 mg/mL	-0.2428	0.5291	-0.3458	0.3620
	10 mg/mL	-0.2076	0.5290	-0.6403	0.0632
MTT	1 mg/mL	-0.03229	0.9343	0.03151	0.9359
	5 mg/mL	-0.6554	0.0553	-0.7275	0.0263*
	10 mg/mL	0.01096	0.9777	0.2356	0.5416
PT	1 mg/mL	-0.2630	0.4942	-0.5689	0.1099
	5 mg/mL	0.4590	0.2140	0.1674	0.6680
	10 mg/mL	0.4771	0.1941	-0.2046	0.5075
TTPa	1 mg/mL	0.2343	0.5441	0.7053	0.0338*
	5 mg/mL	0.1118	0.7745	0.5109	0.1598
	10 mg/mL	-0.1566	0.6874	-0.0997	0.7986

*S. A: Surface area; %A. S: Acid sites. * p < 0.05, ** 0.01

Conclusion

Nine Nb_2O_5 samples were synthesized and evaluated for their characterization and cytotoxicity against erythrocytes, plasma and the ZF-L cell line. While the addition of different surfactants did not significantly alter the morphology and chemical structure, notable changes were observed in the acid sites and surface area, with Nb_2O5 having the highest surface area (240.4 m2/g) and the lowest pore size diameter (2 nm). NbSDS5 reduced by 6-fold the area, whilst NbOA5 increased the pore diameter by 13-fold. Hemolytic activity showed a concentration-dependent behavior, with Nb2O5 and NbCA1 at 1 mg/ml being safe but at 10 mg/ml causing almost 100% of erythrocyte rupture. Anticoagulant activity was maintained intact for the extrinsic pathway, but the intrinsic side showed a delay in coagulation. Interestingly, cytotoxicity assays revealed that cells were able to survive at most concentrations in an acidic pH, with some even showing increased proliferation, as is the case for the 1.3-fold by NbSDS1 at 1 mg/ml. Although Pearson's analysis did not find any linear correlation between surface area, acid sites, and cytotoxicity tests, this is the first study evaluating these parameters not only for pure Nb_2O_5, but for different surface modifications caused by surfactants. Further studies are required to comprehend how these changes influence the material's cytotoxicity properties.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge the support of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES, LAPEBBIOM, Novonano, IC2MP, Research Group on Adsorptive and Catalytic Process Engineering (ENGEPAC). This study was financed in part by CAPES – code 001 and NIOBIUMCAT (CAPES-COFECUB project Ph-C 963/20).

Author contributions

MMC: conceptualization, methodology, investigation; writing-draft, writing- final version; VPG: investigation; LBM: investigation; CJAP: data analysis, final review; FCR: investigation; writing-draft; BNR: investigation; LG: investigation; BVL: investigation; GCC: investigation; writing-draft; CE: supervision, review; GL: supervision, review; LV: supervision, review; NLVR: supervision; funding acquisition, writing-final draft, review; CMPP: supervision, funding acquisition, review; JLG: supervision, review; RAV: supervision, writing-final version, funding acquisition.

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4.3 Capítulo 3

O terceiro capítulo deste trabalho de tese avaliou a atividade *in vivo* de ON estudados anteriormente em um modelo de *D. melanogaster*. Neste trabalho, buscou-se avaliar alguns marcadores bioquímicos, estresse oxidativo e o estudo da modulação a nível gênico, após os dados de triagem obtidos no capítulo 2.

A utilização de *D. melanogaster* ocorreu em consequência da facilidade de manuseio e rápido ciclo de vida. Por se tratar de um modelo invertebrado, este capítulo não necessitou da aprovação do Comitê de Ética em Pesquisa Animal. Contudo, continuou-se empregando os protocolos de segurança e manejo adequados, visando a segurança dos pesquisadores e preservando a ética perante o uso de animais.

Os compostos escolhidos englobaram dois aspectos: o primeiro relaciona-se ao composto que conseguiu diminuir o valor de IC_{50} da atividade hemolítica do ON puro (NbCA1); e o segundo preceito relacionou-se com a escolha de dois compostos com surfactantes de origem diferentes, ou seja, um catiônico (NbOA1) e um aniônico (NbSDS5), e que o valor de IC_{50} não fosse muito extrapolado positivamente.

É importante mencionar que, assim como no capítulo anterior, não foram encontrados estudos avaliando o ON em um modelo de *D. melanogaster*. Alguns estudos visaram observar o comportamento de camundongos *Swiss* machos após uma injeção intraperitoneal de uma suspensão de 3% de ON comercial (Dsouki *et al*., 2014), como também a melhoria de um material prostético a partir da adição de ON (1:1) em Cálcio Fosfato Bifásico utilizando ratos Wistar (*Rattus norvegicus, var. albinus) (Liu; Lun, 2012).* Ambos não encontraram resultados tóxicos nos animais, e o ON não foi efetivo em causar proliferação celular ou melhorias na prótese.

As análises envolvendo colinesterase e a expressão gênica de diferentes marcadores não está anexada ao manuscrito desse capítulo, pois houve atraso no envio dos reagentes pelas empresas responsáveis. Estes serão anexados ao trabalho após a defesa de tese.

O artigo encontra-se em forma de manuscrito preliminar, e será submetido para a revista **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology** (fator de impacto 3.9, qualis A1).

Preliminary *in vivo* oxidative stress and metabolic markers of surface-modified niobium oxides in *Drosophila melanogaster*

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Abstract

Niobium oxide (Nb_2O_5) is emerging as an interesting biomaterial due to its unique physicochemical properties, such as a high corrosion rate, the ability to undergo surface modification, and its inertness. Despite its potential, the biological effects of Nb_2O_5 remain underexplored. In this sense, the aim of this study was to evaluate safety and toxicity of different Nb_2O_5 samples, with or without surface modification from surfactants, on an animal model using *Drosophila melanogaster*. The survival rate was over 80% for all samples after acute exposure for a 72-hour period. Metabolic parameters such as glucose and triglycerides were evaluated, as well as oxidative stress markers, including TBARS, NPSH, and RS. Our results show that NbSDS5 had a significant increase in triglyceride levels from 10x to 50x. NbOA1-50 decreased glucose levels, while NbOA1-10, Nb_2O_5-50, and NbCA1-10 increased it. Our study showed that oxidative stress was increased in NPSH and RS, but not in TBARS, indicating possible protection of the lipid system, may be due to the GSH-GSSH recycling process. This is the first study exploring the *in vivo* modulation of Nb_2O_5.

Keywords: Niobium oxide, *Drosophila melanogaster*, oxidative stress, biochemical markers, gene expression.

1 Introduction

Nanotechnology significantly increased its use in several fields, such as catalysis, electrochemistry and the biomedical area. Among these materials, niobium oxide has gained a great attention due to its physical-chemical properties, as it tends to be insoluble, inert, resistant to corrosion and possesses an interesting surface modification ability (Nico; Monteiro; Graça, 2016b; Safavi *et al.*, 2022). In that sense, it holds great potential for implantable devices (de Almeida Bino *et al.*, 2021b; Miyazaki *et al.*, 2001; Pradhan *et al.*, 2016) and cancer therapy (Sun et al., 2024).

Although promising, the biological effects of niobium oxide remain underexplored. There are several studies that evaluate the use of niobium oxide as a coating material for stainless steel (Ramírez *et al.*, 2011) magnesium (Amaravathy *et al.*, 2014) and titanium (de Almeida Bino *et al.*, 2021) alloys. However, these studies focus on *in vitro* tests. There are only a few studies that evaluated its behavior *in vivo,* but they didn't' find any hematological changes in Swiss mice (Dsouki *et al.*, 2014). Moreover, the National Institute of Health mentions in their safety data sheet that it may cause irritation to the skin and can be harmful for rodents over 4 mg/kg (National Institute of Health, 2025).

In this context, the use of *Drosophila melanogaster* as an experimental model has its advantages due to its cost-effectiveness, rapid development and genetic simplicity. Moreover, it has an interesting homology with the human genome (Baenas; Wagner, 2022; Severino *et al.*, 2023), making it a great candidate for safety and toxicity tests. Studies using this animal can provide insights into how nanomaterials can behave in the organism without using a mammalian model, at least at first. Zinc oxide nanoparticles, for example, were evaluated in *D. melanogaster*, indicating a change in the climbing behavior (Anand *et al.*, 2017) and increase in the oxidative stress markers (Ng *et al.*, 2019). Magnetic iron oxide nanoparticles displayed a mutagenic effect (Güneş et al., 2024), and magnetite nanoparticles reduced the female fecundity (Chen *et al.*, 2015)

The aim of this study was to evaluate the influence of different niobium oxide nanoparticles in a preliminary set of metabolic markers, oxidative stress and genomic alterations in *D. melanogaster*. Our study seeks to improve the *in vivo* understanding and provide future insights for the development of niobium oxide-based biomaterials. Our results show that niobium oxide can interfere with biochemical parameters and alter the reactive species amount in the flies.

2 Materials and methods

Fly strains and culture conditions

Wild-type *Drosophila melanogaster* Harwich strains were kindly provided by the Laboratory of Clinical Analyses (LEAC), under the supervision of Professor Ana Colpo, at the Universidade da Região da Campanha (URCAMP) in Bagé, Rio Grande do Sul, Brazil. The flies were maintained under controlled temperature of 25°C ± 1 °C with a 12/12h dark-cycle photoperiod. A total of eighty adult male flies were used for each concentration. The exposure to the compounds lasted three days (72 h) to assess the acute treatment of the compounds. In this study, only male flies were used as there is less hormonal fluctuation. For sex separation, the flies were anesthetized with ethyl ether (99%).

Diets

The standard diet (SD) was prepared according to previous work described by (Bahadorani *et al.*, 2008), with modifications. Briefly, the SD consisted in 50 mL of water, 2.5 g of dry yeast, 1 mL of fungal agar, 4.5 mL of commercially available sugar, 9.2 mL corn flour, 0.1 mg Nipazol® (antimycotic agent), 0. 25 ml acid solution (1:10 phosphoric acid/acetic acid), added in that specific order. To evaluate the effect of the niobium oxide samples, values consisting of 10x and 50x of the hemolytic activity from our previous research were added in the same proportion to the SD during the preparation of the diet. All tests were performed in duplicates for each concentration, including the SD. Flies were maintained in contact with the diet for a period of 72 h (Figure 1).

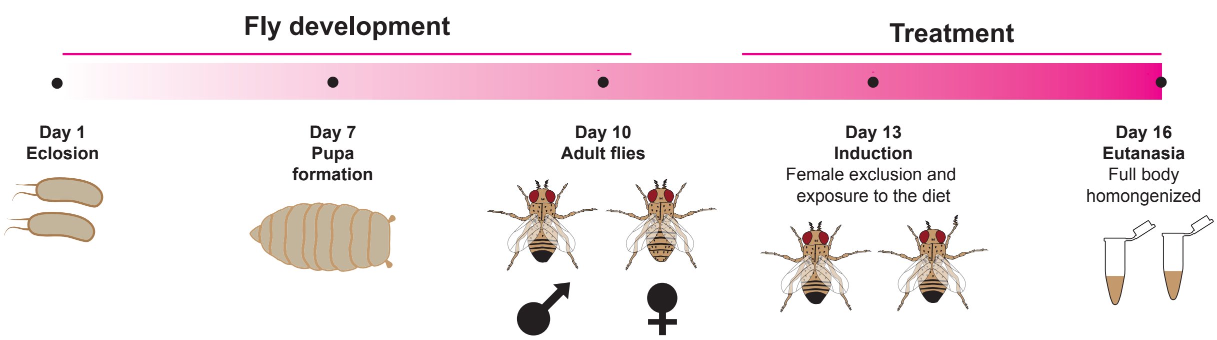

Figure 1: Schematic representation of experimental protocol using *D. melanogaster*.

Survival rate

To evaluate *D. melanogaster's* survival rate, the flies for each treatment were counted on a 24-h period during the acute exposure time (72 h). The total amount of animals used (n=80) for each treatment were separated into four groups of 20 flies, and the survival rate was evaluated based on each independent group.

Metabolic parameters and oxidative stress activity

We used homogenized pools from the whole body to assess the oxidative stress levels and metabolic markers. The number of live flies was counted after the 72-h period, and a total of 50 animals were used for each treatment for the metabolic parameters. Another pool consisting of two groups of 10 flies were separated for the oxidative stress tests. All measurements were performed in duplicates. For each pool, flies were quickly frozen at -20 ^{o}C and macerated with of Phosphate Potassium Buffer (PPB). Subsequently, they were centrifuged for 10 min at 2000 rpm. The supernatant was used for the marker's evaluation.

Biochemical Markers

Cholinesterase, glucose and triglyceride levels were evaluated in the automated Cobas Mira analyzer (Roche®, Switzerland), using commercial kits from Labtest (Minas Gerais, Brazil), following manufacturer's protocols. Results are shown as mean ± SD values of the treated groups (mg dL^{-1}).

Lipid peroxidation

TBARS measurements were employed to indirectly indicate malondialdehyde (MDA) content, an important byproduct of lipid peroxidation. Following the method outlined by (Ohkawa; Ohishi; Yagi, 1979), an aliquot of the supernatant was mixed with a reaction solution containing 0.8% thiobarbituric acid, 8.1% sodium dodecyl sulfate, and acetic acid adjusted to pH 3.4. The mixture was then incubated at 95 °C for 2 hours. Subsequently, absorbance was recorded at 532 nm using a spectrophotometer (Agilent - Gen5 Biotek 800 TS). Results were expressed as nanomoles of MDA per milligram of protein.

Sulfhyhdryl groups detection

NPSH levels were measured to evaluate oxidative damage to thiols and indirectly estimate reduced glutathione (GSH) levels. The quantification of NPSH was performed according to (Ellman, 1959). Briefly, the supernatant was mixed in equal volume with 10% TCA, and the resulting mixture was centrifuged at 900 × g for 10 minutes. The protein pellet was discarded, and the clear supernatant was collected to determine free-thiol (SH) group concentrations. An aliquot of the clear supernatant was then mixed with 1 M potassium phosphate buffer (pH 7.4) and 10 mM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). The colorimetric reaction was then read at 412 nm using a spectrophotometer (Agilent - Gen5 Biotek 800 TS). NPSH levels were expressed as nanomoles of NPSH per gram of tissue.

Reactive Species (RS)

RS levels were determined using a spectrofluorimetric method with the 2',7'-dichlorofluorescein diacetate (DCHF-DA) assay. An aliquot of S1 (10μl) was incubated with 10μl of DCHF-DA (1 mM). The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured to detect intracellular RS. The emission of DCF fluorescence intensity was recorded at 520 nm (with 480 nm excitation) using a Shimadzu RF-5301 PC fluorometer, 15 min after the addition of DCHF-DA. Data were expressed as arbitrary units of fluorescence.

Statistical analysis

Data are reported as mean ± SD. Samples were subjected to the normality test Shapiro-Wilk. For the metabolic activity and oxidative stress analyses we performed one-way ANOVA followed by Dunnet's *post hoc*. Significant differences were set at $p \leq 0.05$.

Results and discussion

As an important prerequisite, we previously reported an extensive characterization of several modified Nb_2O_5 (CERVEIRA *et al.*, 2025). The compounds were chosen based on the IC_{50} hemolytic activity and/or the surfactant's nature. NbCA1 was the only one that showed a decrease in IC_{50}. NbOA1 and NbSDS5 were selected due to their cationic and anionic surface modifications' nature, respectively, to evaluate the differences in behavior based on the surfactant used. To the best of our knowledge, this is the first study evaluating different Nb_2O_5 nanoparticles on *D. melanogaster* for any parameter. In that sense, the discussion compared the results obtained with the findings for different NP.

The survival of the flies was observed during an acute exposure of 72 h (Figure 2) and all groups had a survival rate higher than 80%. Nb_2O_5-10 and NbSDS5-10 showed the highest mean percentage of lost animals (82.2% and 86.3%, respectively). NbCA1-10 had the highest survival rate, at 97.5%. Some flies were lost during the transfer between one tube to another and were considered as dead in the survival rate count. Most of the flies died by staying stuck in the diet, which may be caused by changes in the viscosity of the diet by the compounds, and/or by alterations in the flies' locomotor activity.

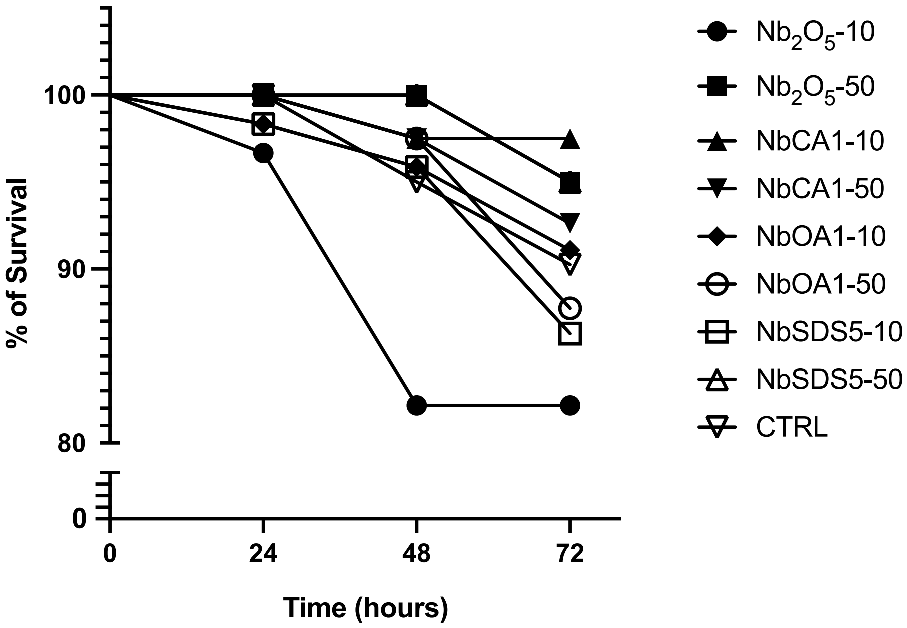

Figure 2: Survival rate of D. melanogaster after an acute treatment of 72h. Flies were counted every 24 h. Data are expressed as percentage from mean of four groups (n=20).

Metabolic parameters (glucose, cholinesterase and triglycerides) were measured from whole body (Figure 3) after an acute exposure to the Nb_2O_5 samples. All tested materials showed a decrease in triglyceride levels with the increase in the amount added to the diet, except for NbSDS5, which highly increased from 10x to 50x. NbSDS5-50 had an increased difference to the control group ($p < 0.05$) while Nb_2O_5-10, Nb_2O_5-50 and NbSDS5-10 displayed a significantly decrease ($p < 0.01$) in triglycerides levels.

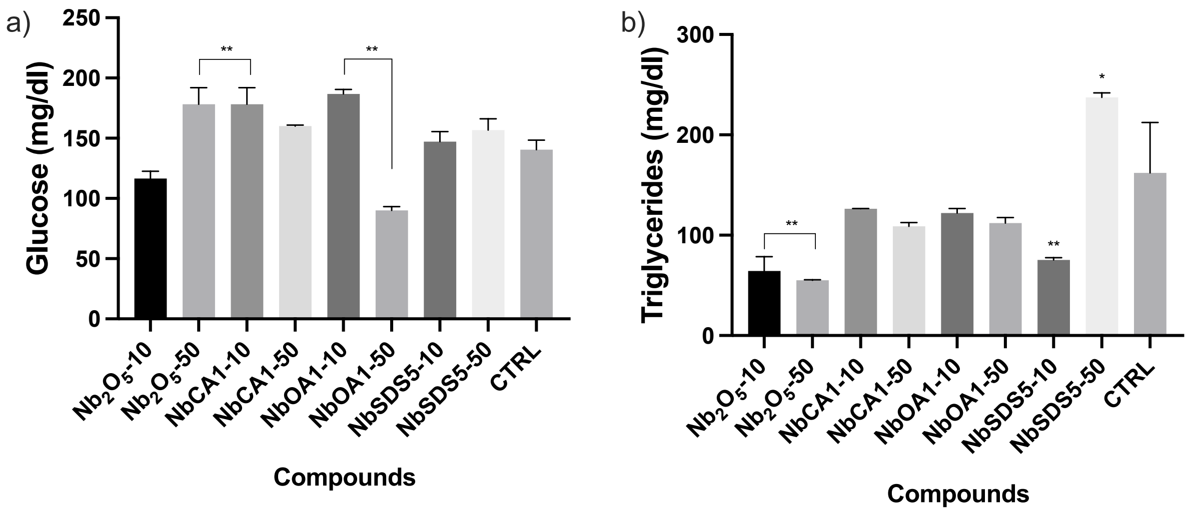

Figure 3: Biochemical parameters from whole body homogenized of *D. melanogaster*. A) Glucose levels, b) triglycerides. Data are expressed as mean +- SD. * $p < 0.05$, ** $p < 0.01$.

Wang *et al.*, 2012 found that AgNP were localized in the lipid droplets, in the fat portion of *D. melanogaster.* Triglycerides were increased by 10% in larvae fed with AgNP, and when exposed to calorie restriction, it was even higher (20%). Raj et al., 2017 showed a reduction in lipid content when exposing the animals to the same NP at 25 mg/L. When blocking the mTOR pathway, researchers were able to prevent fatty acid synthesis caused by AgNPs as well as control the expression of Fatty Acid Synthase and Acetyl-CoA Carboxylase enzymes (Wang et al., 2012). Raj *et al*. (2020) did not find any alterations in the lipid content when exposing the flies to AuNP. Ropinirole silver nanocomposites reduced MDA levels by 2.37-fold, while AgNP did not show any significant changes in a Parkinson's disease (PD) model (Naz *et al.*, 2020).

Flies exposed to TiO_2 displayed a decrease in glucose levels, especially in males (135.8 ± 10.3) and the glucose transport decrease may happen in the gut epithelium (Richter *et al.*, 2018). Raj et al., (2020) also showed a decrease in glycogen with the administration of AuNP, and the downregulation of glycogen synthase. On the contrary, glycogen levels were not altered with the administration of CeO_2 (Sundararajan *et al.*, 2019).

Oxidative parameters were evaluated from different techniques (Figure 4). No statistical differences were observed between the groups that received the SD or different Nb samples in relation to MDA levels. This indicates that RS levels were not significantly influenced by lipid peroxidation. Except for pure Nb_2O_5, the behavior for TBARS and triglycerides were in agreement in our study.

The NPSH assay was performed on whole body flies homogenized. It was observed that different treatments caused an increase in NPSH levels when compared to the control group. All groups showed a small decrease when changing the diet from 10x to 50x, except for NbSDS, which had the opposite behavior. In agreement, the co-treatment of *Tetrapleura tetraptera* along with the exposure of $CdCl_2$ was able to increase the NPSH levels, indicating a protective effect of the extract (Oyibo *et al.*, 2025). Ropinirole silver nanocomposite restored the NPSH content in a PD'S *D. melanogaster* model (Naz et al., 2020).Palm oil intake showed an initial reduction in the NPSH levels, but the levels were recovered in a chronic exposure (da Silva *et al.*, 2024).

Along that, RS levels were also measured in fly tissues. All samples had a slightly increase in RS activity with increased concentration, but not for pure Nb_2O_5. NbOA50, NbSDS10 and NbSDS50 had a higher significance difference ($p < 0.005$) compared to the control group. Our results indicate that different treatments led to a significant increase in RS levels compared to the control group. These findings suggest that these compounds contribute to a higher oxidative stress state.

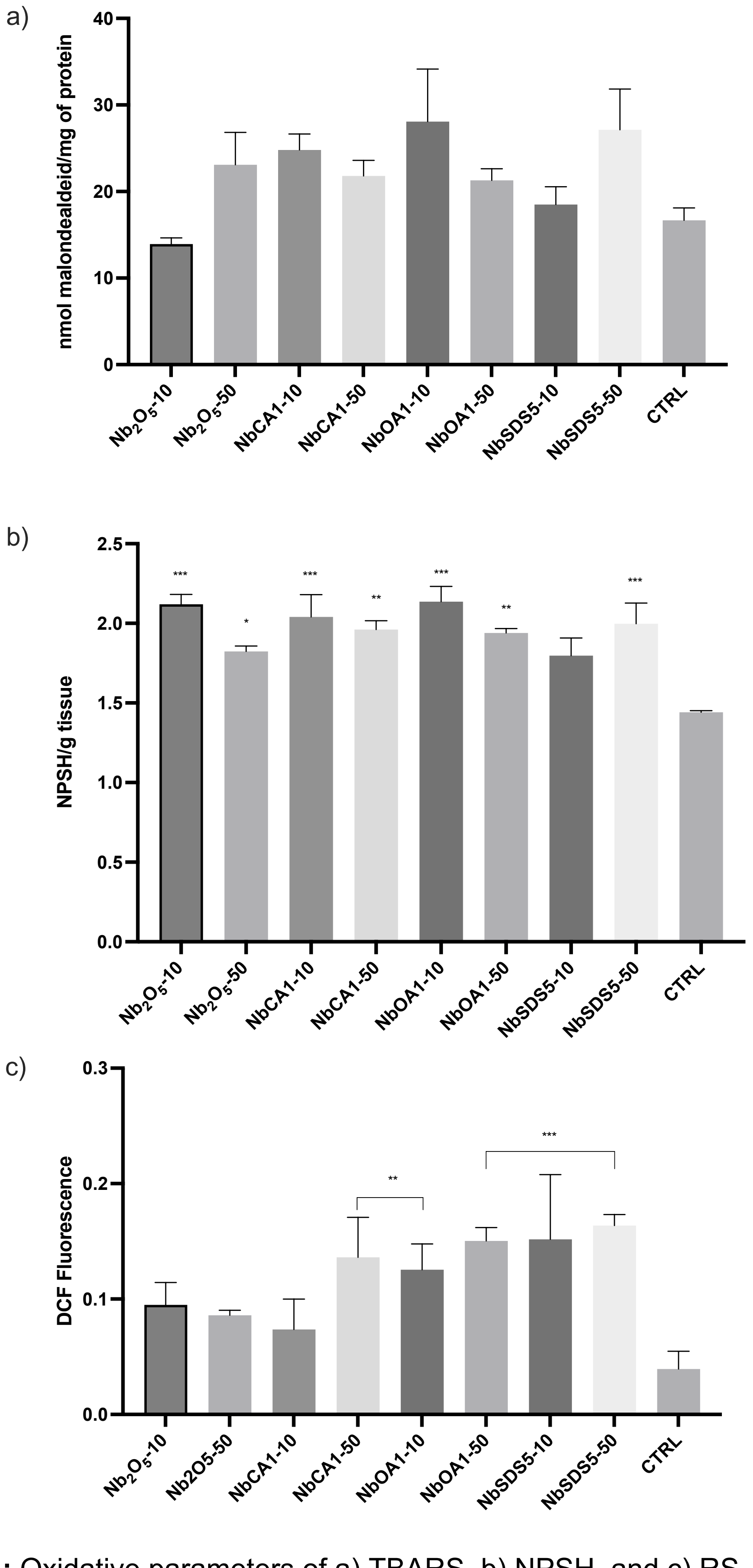

Figure 4: Oxidative parameters of a) TBARS, b) NPSH, and c) RS. Data are expressed as mean +- SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

ROS levels decreased by 48% at 10 µg/ml and 42% at 100 µg/ml for MoS_2, and 25.5% at 10 µg/ml ($P < 0.05$) and 61% at 100 µg/ml for nanoceria-polyethylenimine-MoS_2 ($NCeO_2$-PEI-MoS_2) nanoflakes (Murugan *et al.*, 2022). The authors suggest that pure MoS_2 requires a smaller concentration because it mimics the activity of antioxidant enzymes, while ($NCeO_2$-PEI-MoS_2) needs a higher amount because it has a rapid degradation in the gut. Flies exposed to a 0.5 mg/ml ZnO NP showed a huge increase in ROS levels and hence the gut cell viability (Ng *et al.*, 2019). The production of ROS by AgNPs was also confirmed by Agrawal (2017) using a 25 mg/L concentration, highly localized in the fat body. CoNPs had an increase in ROS production when flies received 2 mM. On the contrary, ROS production in flies exposed to CeO_2 was not altered after a month (Sundararajan *et al.*, 2019).

When evaluating all tested oxidative stress and metabolic parameters, the lipid peroxidation does not seem the main subject being oxidized. With the increased levels of NPSH, we can hypothesize that the recycling of the GSH-GSSH system could be protecting these molecules, while the oxidation is occurring in its majority in other macromolecules. However, further studies are needed to investigate which molecules are more prone to the oxidative stress, and if NADPH-$NADP^+$ is also being affected. Unfortunately, we did not have the structure to perform these tests. The MGA levels, for example, were increased for AgNP exposure, while GSH levels showed the opposite behavior (Ahamed *et al.*, 2010). In male wistar albino rats, the same behavior was observed for the oral ingestion on CuO NP after 14 days (Anreddy, 2018).

A chronic exposure to Nb_2O_5 would also be interesting, as it is known that TiO_2 NP can cause phenotypic alteration in the wings during a long-term exposure (Cvetković *et al.*, 2020). Long-term exposure to ZnNP corroborates with the study, but also showed deformed thorax, passing some of the mutations to the following generation (Anand *et al.*, 2017) Further studies must be conducted in a chronic exposure of Nb samples to evaluate behavior and phenotypic alterations. AgNP were studied during eight fly generations, indicating first a reduction in the fertility. Interestingly, the subsequent flies were able to restore these levels, as they were able to adapt to the NP exposure (Panacek *et al.*, 2011).

Conclusion

This is the first study evaluating the influence of Nb_2O_5 samples in an *in vivo* model of *D. melanogaster.* Our results showed that the compounds were able to modulate some markers, such as glucose and oxidative parameters (NPSH and RS). The non-significance alterations in triglycerides and TBARS may indicate that the fat tissue is being held protected, as it is the main form of energy storage. Apart from being an exclusive research, further studies could be performed for a deeper understanding of the mechanisms by which Nb_2O_5 interferes with these parameters.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge the support of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES, LAPEBBIOM, Novonano, IC2MP, Research Group on Adsorptive and Catalytic Process Engineering (ENGEPAC). This study was financed in part by CAPES – code 001.

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5 DISCUSSÃO

Com base nos resultados apresentados neste trabalho de tese, foi possível estudar as propriedades físico-químicas e as atividades *in vitro* e *in vivo* de diferentes Nb_2O_5 modificados superficialmente. Somado a isso, o Nb_2O_5 puro também foi utilizado em todos os testes, de modo não apenas para servir como controle, mas também devido à literatura ser muito rasa no comportamento deste quando em seu aspecto natural.

No primeiro capítulo, elaborou-se um artigo de revisão, onde se observou que a maior parte da literatura encontrada sobre o Nb_2O_5 está focada na utilização deste como revestimento para diferentes biomateriais, sejam eles metálicos (como o 316 L e o Ti6Al4V), vidros como o Bioglass® ou ainda materiais poliméricos com gelatina e PCL. A maioria dos revestimentos se mostrou promissor para melhorar a qualidade dos biomateriais, como é possível perceber no trabalho de Ramirez e colaboradores (2011), em que o Nb_2O_5 foi capaz de aumentar a diferenciação celular de cementoblastomas, e melhorar o metabolismo de Saos-2 quando incorporado ao Bioglass® (Hammami *et al.*, 2023).

Ainda, foi possível observar que não existe uma padronização quanto à nomenclatura para cada cristalização do Nb_2O_5, pois diferentes autores utilizam diferentes nomes (ou não mencionam qual polimorfo foi utilizado). Essa é uma pauta antiga, visto que a nomenclatura dada por Schafer e colaboradores em 1966 não foi amplamente aceita, como já discutido por Nico e colaboradores (2016). Ainda que as temperaturas de transição de fases não sejam específicas, pois dependem de muitos parâmetros durante a síntese, sabe-se que a identificação da cristalinidade é essencial para tentar compreender o comportamento do material, como já demonstrado que apenas a variação da temperatura de calcinação pode alterar a viabilidade celular em uma linhagem de L929 (Pradhan et al., 2016).

O primeiro capítulo demonstrou também que há uma lacuna muito grande no entendimento do Nb_2O_5 isolado, sendo poucos estudos encontrados na literatura, mesmo que apresente uma certa eficácia quando utilizado como revestimento, tanto na corrosão do material (Eduok, 2020; Ramírez *et al.*, 2011) quanto na viabilidade celular (Hammami *et al.*, 2023; Pradhan; Wren; Mellott, 2017; Young *et al.*, 2014). Contudo, não se sabe exatamente qual é o comportamento caso o Nb_2O_5 seja liberado na corrente sanguínea, ou como ele interage com outras biomoléculas. Sabe-se apenas que o Nb_2O_5 é capaz de afetar as albuminas séricas bovina e humana, alterando as conformações secundárias e terciárias das mesmas (Millan; Susrisweta; Sahoo, 2023). O Nb_2O_5 também foi capaz de causar danos ao DNA em baixas concentrações (53, 105, e 210 µg/mL para o material cristalino e 210 µg/mL para o ON amorfo), e ainda gerou danos cromossomais (Schardosim *et al.*, 2022). Diante disso, observou-se a necessidade de estudar o ON isoladamente e causar modificações superficiais para buscar entender se há alguma alteração na citotoxicidade.

A modificação superficial com surfactantes é uma alternativa para modular o tamanho da NP, uma vez que tende a impedir a aglomeração. Pode, ainda, adicionar grupos funcionais à superfície (Shaban; Kang; Kim, 2020). Os resultados obtidos no capítulo 2 pela adição de diferentes surfactantes *in situ* na produção de Nb_2O_5 pela síntese de micro-ondas não foi efetiva para a adição de grupos funcionais, mas alterou parâmetros como área superficial e sítios ácidos. A síntese dos materiais seguiu o protocolo de Ücker e colaboradores (2019) e foi realizada previamente à produção desta tese. A síntese de *nanorods* de ON já foi previamente realizada por Gates e colaboradores (2017), os quais observaram diferenças no comprimento das partículas a depender da natureza do surfactante utilizado, que podem levar a alterações na atividade biológica do ON (Pisárčik *et al.*, 2018). As diferentes cristalizações e o tipo de síntese, portanto, vão afetar diretamente o comportamento final do ON, principalmente ao utilizá-lo como um biomaterial.

Em relação à citotoxicidade, não foram encontrados na literatura dados que avaliem a atividade hemolítica e em células hepáticas do Nb_2O_5, sendo, portanto, este trabalho de tese um estudo inédito. Os eritrócitos, por exemplo, estão presentes em grande quantidade no organismo e executam funções essenciais, como o transporte de oxigênio, remoção de gás carbônico e regulação do pH sanguíneo (Morera; Mackenzie, 2011). Apenas um estudo foi encontrado avaliando alterações hematológicas do Nb_2O_5 em camundongos *Swiss*, utilizando uma suspensão a 3% em tampão PBS por via intraperitoneal, mas sem resultados negativos (Dsouki *et al.*, 2014). Os hepatócitos, por outro lado, são importantes por serem as principais células do fígado, sendo este o órgão que está relacionado com a metabolização de moléculas, síntese de proteínas e desintoxicação por xenobióticos. São, portanto, cruciais para a manutenção da homeostase, ao regular a produção da bile e contribuir para respostas imunológicas (Dutta et al., 2021; Zhang et al., 2024).

Concomitante a isso, a avalição de sítios ácidos é de grande interesse para a catálise (Nowak; Ziolek, 1999). A partir do entendimento da acidez de um material, diferentes aplicações podem ser abordadas para uma reação mais efetiva. Ainda que não muito utilizada na área farmacológica, essa técnica pode levar a uma contribuição em estudos que avaliem a interação de um material com certos grupos funcionais e suas cargas específicas, como diferentes macromoléculas e/ou micro-organismos, visto que estes também apresentam uma carga própria (Goldenberg; Steinberg, 2010). Compreender a interação eletrostática e/ou química de ambos pode abrir novos caminhos para o entendimento de como o material se comporta no organismo.

A interação entre o Nb_2O_5 e os eritrócitos, por exemplo, os quais tem também sua superfície carregada negativamente, pode ocorrer através da neutralização das cargas negativas por prótons ou outros cátions presentes no meio. Essa interação dos prótons, característico de um ácido de Brönsted, tende a gerar uma alteração no pH e, consequentemente, alterar a estrutura da membrana ou das proteínas presentes na célula (Murador; Deffune, 2007). O efeito hemolítico no capítulo 2 demonstrou que baixas concentrações podem não causar um efeito tão notório, mas o aumento para 5 e 10 mg intensificou esse comportamento. Os sítios ácidos, ainda que não tenha sido identificada nenhuma correlação linear com a atividade hemolítica, demonstra que o NbCTAB5 foi o composto com menor hemólise, e com menos sítios ácidos fortes (> 445 ^{o}C). Já o NbCTAB1 teve a menor viabilidade a 1 mg/ml, e possuindo o maior valor com sítios ácidos muito fracos (100-260 ^{o}C).

Ademais, um dos processos essenciais para manter a homeostase sanguínea é a coagulação, além de seu papel no sistema imune (Lozano-Fernández *et al.*, 2019). Dessa forma, o estudo da interferência de NP metálicas é essencial para a compreensão, juntamente com a atividade hemolítica, do comportamento desses materiais na corrente sanguínea. Os Nb_2O_5 avaliados neste estudo foram capazes de interferir, em grande maioria, na via intrínseca, aumentando o tempo de coagulação no teste de TTPa. Em vários casos, não houve coagulação mesmo após 120 segundos. Os resultados corroboraram com outras NP como TiO_2 e ZnO (I e II), as quais também induziram um atraso no tempo de TTPa (Lozano-Fernández *et al.*, 2019). O ZnO também interfere na conformação espacial do fibrinogênio, o que altera as interações e causa uma ligação mais fraca. O Nb_2O_5 causa modificações na albumina, a proteína mais abundante no plasma (Millan; Susrisweta; Sahoo, 2023), o que pode estar relacionado com as vias de coagulação, visto que pode alterar a viscosidade e pressão oncótica. Essas NP já são largamente utilizadas em produtos do dia a dia, como em protetores solares e pigmento para cosméticos, que podem entrar em contato com a corrente sanguínea.

A citotoxicidade em relação aos hepatócitos de *Danio rerio* pode ser também ser observada, gerando um efeito qualitativo ao contrário de um quantitativo. Isso pode ocorrer pela interação específica em um ou mais receptores ou proteínas, ou responder apenas ao estímulo inicial para desencadear uma sinalização celular, como ocorre com os receptores de superfície de ácidos graxos livres, que não dependem de variação de dose (Al Mahri *et al.*, 2022). O Nb_2O_5 pode também se comportar como o ácido oxálico, o qual sequestra íons de cálcio para a formação de cristais, alterando a função hepática (de Caldas Brandão Filho *et al.*, 2022). Dessa forma, estudos mais específicos devem ser realizados para melhor entender o comportamento do Nb_2O_5 dentro das células.

Os Nb_2O_5 podem, em teoria, se comportar como ácidos de Lewis, interagindo com grupos funcionais presentes em proteínas, possivelmente pela interação com o Nb, uma vez que este pode adquirir diferentes estados de oxidação; podem também agir como ácido de Brönsted, doando prótons e alterando o pH do meio. O Nb_2O_5 também poderia atuar como ambos os tipos de ácido sendo concomitante o seu papel na doação de prótons e na interação com os grupos funcionais (Foo *et al.*, 2014; Lebarbier; Houalla; Onfroy, 2012; LibreTexts Chemistry, [*s. d.*]). Contudo, estudos mais aprofundados são cruciais para esse entendimento, como a interação do Nb_2O_5 com o Ca^{+2}, cofator da cascata de coagulação e necessário para os hepatócitos, e com o grupo heme, por exemplo.

Ademais, a presença de Nb no meio intracelular ainda não é bem compreendida. Não foram encontradas muitas referências sobre a dissociação do Nb a partir do Nb_2O_5 e se esse comportamento pode gerar toxicidade no organismo. A citotoxicidade em hepatócitos foi avaliada neste estudo utilizando o sobrenadante resultante da exposição do meio utilizado para as células durante 24h, além da avaliação da presença de íons de Nb na solução. Ainda que a presença de Nb não tenha sido diretamente relacionada à citotoxicidade, foi possível observar a alteração de pH do meio para amarelo a partir da mudança de coloração do indicador fenol vermelho. Sabe-se que, abaixo de pH 6, esse indicador se torna amarelo (Weiskirchen *et al.*, 2023). De forma interessante, as células foram capazes de sobreviver mesmo em situações adversas com o pH ácido e, em alguns casos, como para NbSDS1 a 1 mg/ml, até induzir a proliferação celular. O comportamento contrário foi observado para NbOA5 a 10 mg/ml, com apenas 13,25% de viabilidade celular. Essa alteração de pH pode ter sido causada pela acidez do Nb_2O_5. Entretanto, ainda que com a perturbação do meio, o Nb_2O_5 aparenta interferir também em outros fatores, visto que houve proliferação celular em um ambiente hostil.

São poucos também os estudos que envolvem a avaliação do Nb_2O_5 *in vivo*. O conteúdo de Nb no organismo encontrado foi de 100 mg nos anos 70, possuindo afinidade pelo tecido adiposo, fígado e baço. A presença no tecido adiposo de NP metálicas foi estudada também por Song e colaboradores (2012), os quais observaram que AgNPs estavam, em grande maioria, nos *droplets* de lipídio de *D. melanogaster.* Barry e colaboradores (1993) ainda mostraram a presença de Nb nos lisossomos utilizando um Nb radioativo (^{95}Nb), e que esse envolvimento pode estar relacionado com a enzima fosfatase ácida, a qual hidrolisa grupos fosfatos na presença de ambientes ácidos (Anand; Srivastava, 2012). Um estudo envolvendo cloreto de Nb ($NbCl_5$) demonstrou que não houve alteração em eritrócitos de ratos (não houve identificação da raça) em uma exposição crônica e, nos órgãos, o único afetado foi o fígado. Estes animais apresentaram colapso instantâneo na dose de 5 mg/kg e parada respiratória, sendo a dose letal necessária para matar 50% dos animais (LD_{50}) em 7 dias foi de 61 mg/kg (Haley; Komesu; Raymond, 1962).

A utilização do modelo animal *D. melanogaster* buscou suprir uma lacuna da literatura referente a utilização de Nb_2O_5 em um modelo *in vivo*. De forma preliminar, foi possível verificar a modulação de marcadores como glicose e triglicerídeos. Alguns estudos já avaliaram positivamente este modelo para o estudo de comorbidades como a diabete mellitus ((Álvarez-Rendón; Salceda; Riesgo-Escovar, 2018; Baenas; Wagner, 2022) e a obesidade (Brookheart; Duncan, 2016; de Paula *et al.*, 2018), visto que o mecanismo bioquímico e os genes de interesse são bem conservados. Mesmo em uma administração aguda, os Nb_2O_5 alteraram os valores desses marcadores. Estudos futuros envolvendo uma administração crônica são essenciais para compreender de que forma o Nb_2O_5 pode causar complicações a longo prazo, e de que forma a administração desses compostos pode impactar em modelos de comorbidades, tanto a nível bioquímico quanto comportamental.

Os dados de medição de colinesterase ainda não estão totalmente finalizados, por se tratar de um manuscrito não finalizado, mas foi possível observar uma diminuição drástica nos valores desse marcador. Dessa forma, pode-se aferir que os

ON foram capazes de alterar a locomoção dos animais. A colinesterase é composta por duas enzimas: a acetilcolinesterase (AChE) e a butirilcolinesterase (BChE). Embora possuam funções complementares, ambas atuam no processo de modulação do neurotransmissor, o qual participa de sinapses colinérgicas tanto no sistema nervoso central como no periférico. Essa atuação é essencial para funções cognitivas e que podem sofrer degenerações ao longo da vida (De A Cavalcante *et al.*, 2018; Šinko *et al.*, 2014).

Mesmo que neste estudo não tenha sido avaliada a parte comportamental, observou-se durante a finalização do experimento que alguns grupos, em especial o NbSDS5, apresentaram animais mais lentos e com uma maior dificuldade de realizar a escalada nos frascos. NP com quitosana, por exemplo, diminuíram os níveis de AChE em *D. melanogaster*, ao mesmo tempo em que aumentaram o número de animais mortos, mesmo sem nenhuma alteração nos testes comportamentais (Machado *et al.*, 2024). AgNPs se mostraram inibidores reversíveis dessas ezimas (Šinko *et al.*, 2014). O inseticida clorpirifós reduziu os níveis de AChE e a atividade locomotora em *D. melanogaster* (Rodrigues *et al.*, 2019)

Ademais, a modulação de marcadores de estresse oxidativo indicaram que não houve uma alteração significativa no índice de peroxidação lipídica, avaliado através do ensaio de TBARS; contudo, observou-se que os níveis de NPSH e RS obtiveram diferenças significativas. Isso pode indicar uma renovação do sistema de NPSH, de forma que a glutationa (GSH) está sendo renovada após a sua conversão em dissulfeto (GSSH), permitindo a proteção do sistema lipídico. Esses dados podem se correlacionar com os níveis de triglicerídeos, os quais também não foram afetados significativamente. A compreensão futura dessa modulação pode trazer esclarecimentos sobre a influência dos ON no estresse oxidativo e na reciclagem de GSH (Cassier-Chauvat *et al.*, 2023; Lacher *et al.*, 2015; Zhang *et al.*, 2018) .

Entretanto, com os resultados futuros que serão obtidos a nível molecular, e que finalizarão o terceiro capítulo, pretende-se ter uma compreensão maior sobre a modulação causada pelos Nb_2O_5. Com os resultados dessas análises, será possível correlacionar os dados já obtidos a nível bioquímico com as expressões de diferentes marcadores moleculares, podendo explicar tanto uma parte do perfil oxidativo como a possível modulação de marcadores inflamatórios. A compreensão dessas alterações pode auxiliar o planejamento de novas exposições do Nb_2O_5 e da necessidade de se avaliar outros parâmetros.

De modo geral, os estudos dessa tese foram eficazes para a) compreender a literatura de forma geral sobre a utilização do Nb_2O_5 na área biomédica, como explicitado no capítulo 1; b) caracterizar Nb_2O_5 e de modificações superficiais a partir de uma síntese de micro-ondas, buscando estudar também uma triagem citotóxica destes compostos em células como eritrócitos, hepatócitos e na cascata de coagulação sanguínea, além de avaliar a dissociação do Nb a partir do Nb_2O_5; e c) compreender a influência do Nb_2O_5 em um modelo *in vivo*, utilizando *D. melanogaster*, em marcadores bioquímicos e de estresse oxidativo. Os resultados expostos, ainda que definitivos, podem sofrer uma intervenção e serem reanalisados de forma diferente e/ou complementar, garantindo a correta compreensão dos mesmos. Espera-se que, com os resultados pendentes, este projeto possa não apenas suprir uma deficiência da literatura em relação aos compostos estudados, mas também permitir novos estudos em buscar de um melhor entendimento da presença de Nb e do Nb_2O_5 no organismo.

6 CONSIDERAÇÕES FINAIS

Com base nos resultados e discussões apresentados, fica evidenciado que a adição de surfactantes na síntese de Nb_2O_5 não foi capaz de causar adições de grupos funcionais na superfície das partículas, mas foi efetivo em diminuir a área superficial, e alterar os valores de sítios ácidos. O Nb_2O_5, ainda apresenta citotoxicidade eritrócitos, principalmente a 10 mg/ml e, em determinadas concentrações, um aumento na proliferação celular em células hepáticas, mesmo em pH ácido (< 6). Ainda, observou-se que estes interferem na via intrínseca da cascata de coagulação, e que houve extravasamento de Nb a partir de Nb_2O_5 no meio de cultura. Ademais, percebe-se uma modulação de marcadores bioquímicos, como triglicerídeos e glicose em um modelo de ingestão oral aguda (72h) utilizando *D. melanogaster*. A elaboração de um artigo de revisão permitiu observar as lacunas existentes na literatura e que serviram de base para a realização deste projeto de tese. Além destes fatores, os tratamentos causaram aumento no estresse oxidativo dos animais, mas com uma possível proteção do sistema adiposo, visto que os níveis de TBARS não foram alterados significativamente, ou ainda que os níveis de NPSH podem estar compensando o aumento dos níveis de RS iniciais. Assim, conclui-se que o Nb_2O_5 foi efetivo em causar modulações tanto nos *estudos in vitro* quanto *in vivo*, evidenciando ainda mais a necessidade de estudos mais aprofundados sobre como o Nb_2O_5 atua no organismo.

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8 ANEXOS

Anexo I – Comprovante de submissão do manuscrito científico do Capítulo 1

Gmail Milena Cerveira <cerveiramm@gmail.com>

BioMetals - Receipt of Manuscript 'Exploring the Application...'

1 message

BioMetals <mahalakshmi.palani.1@springernature.com> Mon, Oct 21, 2024 at 12:05 AM
To: cerveiramm@gmail.com

Ref: Submission ID e5f37d4f-43bc-411b-b0fd-687562260008

Dear Dr Cerveira,

Please note that you are listed as a co-author on the manuscript "Exploring the Application of Inorganic Chemistry: Niobium Oxide as a Versatile and Promising Biomaterial", which was submitted to BioMetals on 21 October 2024 UTC.

If you have any queries related to this manuscript please contact the corresponding author, who is solely responsible for communicating with the journal.

Kind regards,

Editorial Assistant
BioMetals

Anexo II – Comprovante de submissão do manuscrito científico do Capítulo 1

Gmail Milena Cerveira <cerveiramm@gmail.com>

Your submission to Biomed. Mater.: BMM-106859

1 message

Biomedical Materials <onbehalfof@manuscriptcentral.com> Sun, Dec 15, 2024 at 11:45 PM
Reply-To: bmm@ioppublishing.org
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Dear Dr VAUCHER,

Re: Structural, Morphological, and Cytotoxicity Evaluation of Surfactant-Modified Niobium Oxides

Manuscript reference: BMM-106859

Thank you for submitting your Paper to Biomedical Materials.

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Yours sincerely,

On behalf of:
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