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# SELÇUK ÜNİVERSİTESİ FEN FAKÜLTESİ FEN DERGİSİ

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# SELÇUK ÜNİVERSİTESİ FEN FAKÜLTESİ FEN DERGİSİ YAYIN İLKELERİ

**Makaleler**, A4 (210 mmx297 mm) boyutunda 12 punto Times New Roman yazı tipinde ve çift satır aralıklı yazılmalıdır. Sayfanın sağında, solunda, altında ve üstünde 2.5'er cm boşluk bırakılmalı ve yazılar sağa–sola dayalı olmalıdır. Makalenin her sayfası ve satırları numaralandırılmalıdır. Yazar ad(lar)ı açık olarak yazılmalı ve akademik unvan belirtilmemelidir. Türkçe hazırlanan makaleler Türk Dil Kurumu'nun son yazım kılavuzu dikkate alınarak yazılmalıdır.

**Makale:** Türkçe Başlık, Türkçe Öz, Anahtar Kelimeler, İngilizce Başlık, İngilizce Abstract, İngilizce Keywords, Giriş, Materyal ve Metot, Araştırma Sonuçları, Tartışma, Teşekkür (varsa), 6. Kaynaklar bölümlerinden oluşmalıdır. Bölüm adları koyu yazılmalıdır. Varsa her bir şekil ve tablolar makale içerisinde bahsedildikleri yerden sonra sırayla yerleştirilmelidir. Makale sonunda; Araştırmacıların Katkı Oranı beyanı, varsa Destek ve Teşekkür Beyanı, Çatışma Beyanına yer verilmelidir.

**Başlık:** Kısa ve açıklayıcı olmalı, 14 punto ve koyu, kelimelerin ilk harfi büyük olmalı, ortalanarak yazılmalı ve 15 kelimeyi geçmemelidir. İngilizce başlık Türkçe başlığı tam olarak karşılamalı, 14 punto ve koyu yazılmalıdır.

**Öz:** Türkçe ve İngilizce özlerin her biri 300 kelimeyi geçmemelidir. Türkçe ve İngilizce özlerde sırasıyla "Öz" ve "Abstract" kelimeleri kullanılmalıdır. Öz, çalışmanın amacını, nasıl yapıldığını, sonuçları ve sonuçlar üzerine yazar(lar)ın yaptığı değerlendirmeleri içermelidir. Öz ve Abstract kısımlarında kesinlikle referans kullanılmamalıdır.

**Anahtar Kelimeler:** Özlerin 1 satır altına, her anahtar kelimenin ilk harfi büyük diğerleri küçük harflerle, mümkünse başlıkta kullanılmayan, çalışmayı en iyi biçimde tanımlayacak en fazla 6 anahtar kelime yazılmalıdır.

**Giriş:** Bu bölümde; çalışma konusu, gerekçesi, konu ile doğrudan ilgili önceki çalışmalar ve çalışmanın amacı verilmelidir.

**Materyal ve Metot:** Bu bölümde; makalede kullanılan materyal ve metot açıkça belirtilmelidir.

**Araştırma Sonuçları:** Elde edilen sonuçlar verilmeli, gerekirse çizelge, şekil ve grafiklerle desteklenerek bulgular açıklanmalıdır. Elde edilen bulgular tekrardan kaçınılması amacıyla ya çizelge ya da grafik olarak verilmelidir. İstatistikî olarak önemli bulunan faktörler, uygulanan istatistik analiz tekniğine uygun karşılaştırma yöntemi ile yorumlanarak ilgili istatistikler üzerinde harflendirme yapılmalıdır. İstatistiki analiz yönteminin doğru seçilmediği ve/ya analizin gereği gibi yapılmadığı durumlarda editörler kurulu makaleyi değerlendirme dışında tutabilir.

**Tartışma:** Bulgular çalışma ile ilgili güncel makalelerle tartışılmalı, ancak gereksiz tekrarlardan kaçınılmalıdır. Bulguların başka araştırmalarla benzerlik ve farklılıkları verilmeli, nedenleri açıklanmalıdır.

**Teşekkür:** Mümkün olduğunca kısa olmalı ve yapılan katkı ifade edilerek verilmelidir.

**Yazar Katkıları:** Şeffaflık için yazarları, ilgili CRediT rollerini kullanarak makaleye bireysel katkılarını özetleyen bir yazar beyanı dosyası göndermeye teşvik ediyoruz: Kavramsallaştırma; Veri iyileştirme; Resmi analiz; Finansman alımı; Soruşturma; Metodoloji; Proje Yönetimi; Kaynaklar; Yazılım; Yönetim; Doğrulama; Görselleştirme; Roller/Yazma - orijinal taslak; Yazma - gözden geçirme ve düzenleme. Yazarlık katkıları, önce yazarların isimleri ve ardından CRediT rol(ler)i ile biçimlendirilmelidir.

**Çıkar Çatışması Beyanı:** Bir gönderinin tüm yazarları adına ilgili sorumlu yazar, çalışmalarını uygunsuz bir şekilde etkileyebilecek (önyargılı) olabilecek diğer kişi veya kuruluşlarla olan her türlü mali ve kişisel ilişkiyi açıklamalıdır.

**Kaynaklar:** Atıflar ve kaynakçanın Endnote programı ile hazırlanması gerekmektedir. Dergimize ait endnote stil dosyaları, Türkçe ve İngilizce makaleler için ayrı olarak dosya yükleme aşamasında indirme linkleri ile paylaşılmıştır.

Kaynaklar listesi yazılırken, birinci yazar Soyadına göre alfabetik sıralanmalı, ilk satırdan sonraki satırlar 1.0 cm sağdan başlamalıdır. Aynı yazar/yazarların farklı eserleri eski tarihliden başlayarak, aynı tarihli eserler tek yazarlıdan başlayarak sıralanmalıdır. Kaynaklar, mümkün olduğunca orijinal dilinde sunulmalıdır. Orijinal dilinde verilemeyen kaynaklar, Türkçe veya İngilizce olarak verilebilir. Ancak bu durumda kaynağın orijinal dili parantez içerisinde belirtilmelidir.

• Kaynak bir makale ise: Yazarın soyadı, adının baş harfleri., yılı, makalenin başlığı, derginin adı (italik), cilt numarası (varsa no ), sayfa aralığı.

Özgören, M., 2006, Flow Structure in the downstream of square and circular cylinders, Flow Measurement and Instrumentation, 17 (4), 225-235.

• Kaynak bir kitap ise: Yazarın soyadı, adının baş harfi(leri)., yılı, kitabın adı, cilt numarası, varsa editör(ler) / çeviri editörleri, yayınlayan yer (italik), yayınlandığı yer, sayfa aralığı.

Dasgupta, D., 1998, Artificial immune systems and their applications, Springer-Verlag, Berlin - Heidelnerg, 45-52.

Not: Çeviri kitaplarda orijinal kitabın değil çeviri kitabın yayın tarihi esas alınacaktır.

• Kaynak basılmış tez ise: Yazarın soyadı, adının baş harfi(leri). (yılı), Tezin adı", Tezin Cinsi (Yüksek lisans/doktora), Tezin Sunulduğu Enstitü (italik), sunulduğu yer, sayfa aralığı.

• Kaynak kongreden alınmış ise: Yazarın soyadı, adının baş harfi(ler)., yılı, Tebliğ Adı, kongre, seminer veya konferansın adı (italik), yapıldığı yer, bildiri kitabında yer aldığı sayfa aralığı.

Güneş, S. ve Polat, K., 2009, Elektrokardiyogram (EKG) aritmi teşhisinde en az kareli destek vektör makinaları kullanımına dayalı medikal teşhis destek sistemi, 13. Biyomedikal Mühendisliği Ulusal Toplantısı, BİYOMUT-2009, İstanbul, 170-173.

• Kaynak rapordan alınmış ise: Yazarın soyadı, adının baş harfi(leri) (raporu hazırlayan tüzel kişi ise kuruluşun adı), yılı, raporun adı, raporu hazırlayan kuruluşun kısa adı ve rapor numarası (italik), yayınlandığı yer (italik), sayfa aralığı.

De Castro, L. N. and Von Zuben, F. J., 2000, Artificial immune systems: Part I- Basic theory and applications, DCA-RT 02/00, Brasil, 23-28.

• Kaynak aktüel dergi ve gazete haberinden alınmış ise:

Corliss, R., 1993, Pacific Overtures Times, 142 (11), 68-70.

• Kaynak yazarı bilinmeyen ulusal bir çalışmadan alınmış ise:

Anonim, 2006, Tarım istatistikleri özeti, DİE Yayınları, No;12, Ankara, 22-23.

• Kaynak yazarı bilinmeyen yabancı bir çalışmadan alınmış ise:

Anonymous, 1989, Farm accountancy data network, an A-Z of methodology, Commission Report of the EC, Brussels, 16-19.

• Eğer aynı yazarın aynı yılda basılmış birden fazla yayını kullanılmışsa basım yıllarının sonuna alfabetik bir karakter ilave edilir. Örneğin aynı yazarın (ların) 2003 yılındaki üç yayını için (2003a, 2003b, 2003c) şeklinde gösteriniz.

• Haritalar için gösterim

Yazarın soyadı, adının baş harf(ler)i., yılı, Başlık, Ölçek, Basım Yeri:Yayınevi.

Mason, J., 1832, Map of the countries lying between Spain and India, 1:8.000.000, London: Ordnance Survey.

• Web sayfaları için gösterim

Yazarın soyadı, adının baş harf(ler)i., yılı, Başlık [online], (Edition), Yayın Yeri, Web adresi:URL [Ziyaret Tarihi].

Holland, M., 2002, Guide to citing Internet sources [online], Poole, Bournemouth University,http://www.bournemouth.ac.uk/library/using/guide_to_citing_internet_sourc.html [Ziyaret Tarihi: 4 Kasım 2002].

**Şekiller ve Tablolar:** Şekil, grafik, fotoğraf ve benzerleri "Şekil", sayısal değerler ise "Tablo" olarak belirtilmelidir. Tüm şekil ve tablolar makalenin içine yerleştirilmelidir. Şekil ve tabloların boyu tek sayfa düzeninde en fazla 16x20 cm ve çift sütun düzeninde ise genişliği en fazla 8 cm olmalıdır. Şekil ve tabloların boyutu baskıda çıkabilecek çözünürlükte olmalıdır. Araştırma sonuçlarını

destekleyici nitelikteki resimler 600 dpi çözünürlüğünde "jpg" formatında olmalıdır. Her tablo ve şekle metin içerisinde atıf yapılmalıdır. Tüm tablo ve şekiller makale boyunca sırayla numaralandırılmalıdır (Tablo 1 ve Şekil 1). Tablo ve şekil başlıkları ve açıklamaları kısa ve öz olmalıdır. Şekil ve tablo başlık yazıları 10 punto, şekil ve tabloların içindeki yazılar 9 punto, tablo altı yazılar 8 punto Times New Roman yazı karakterinde olmalıdır. Tablo ve şekillerde kısaltmalar kullanılmış ise hemen altına bu kısaltmalar açıklanmalıdır.

**Birimler:** Tüm makalelerde SI (System International d'Units) ölçüm birimleri kullanılmalıdır. Ondalık kesir olarak nokta kullanılmalıdır (1,25 yerine 1.25 gibi). Birimlerde "/" kullanılmamalı ve birimler arasında bir boşluk verilmelidir (m/s yerine $m\ s^{-1}$, J/s yerine $J\ s^{-1}$, kg m/s$^2$ yerine $kg\ m\ s^{-2}$ gibi). Sayı ile sembol arasında bir boşluk bırakılmalıdır ( $4\ kg\ N\ ha^{-1}$, $3\ kg\ m^{-1}\ s^{-2}$, 20 N m, $1000\ s^{-1}$, 100 kPa, 22 °C gibi). Bu kuralın istisnaları düzlemsel açılar için kullanılan derece, dakika ve saniye sembolleridir (°, ′ ve ″). Bunlar sayıdan hemen sonra konmalıdır (10°, 45', 60" gibi). Litrenin kısaltması "l" olarak belirtilmelidir. Cümle sonunda değillerse sembollerin sonuna nokta konulmamalıdır (kg. değil kg).

**Formüller:** Formüller numaralandırılmalı ve formül numarası formülün yanına sağa dayalı olarak parantez içinde gösterilmelidir. Formüllerin yazılmasında Word matematik işlemcisi kullanılmalı, ana karakterler 12 punto, değişkenler italik, rakamlar ve matematiksel ifadeler düz olarak verilmelidir. Metin içerisinde atıf yapılacaksa "Eşitlik 1" biçiminde verilmelidir (…ilişkin model, Eşitlik 1' de verilmiştir).

# SELÇUK UNIVERSITY JOURNAL OF SCIENCE FACULTY INSTRUCTIONS FOR AUTHORS

Manuscripts should be written in A4 (210 mmx297 mm) size, 12-point Times New Roman font, and double-spaced. There should be 2.5 cm margins on the right, left, bottom, and top of the page, and the texts should be right-left aligned. Each page and line of the manuscript should be numbered. The name(s) of the author should be written clearly and the academic title should not be stated. Manuscripts prepared in Turkish should be written by taking into account the latest spelling guide of the Turkish Language Association.

**Manuscript:** It should consist of Turkish Title, Turkish Abstract, Keywords, English Title, English Abstract, English Keywords, Introduction, Material and Method, Research Results, Discussion, Acknowledgments (if any), 6. References. Section names should be written in bold. If any, each figure and table should be placed in order after the place they are mentioned in the manuscript. At the end of the manuscript; a Statement of Contribution of the Researchers, a Statement of Support and Acknowledgment, if any, Statement of Conflict should be included.

**Title:** It should be short and descriptive, in 14 points and bold, the first letter of the words should be capitalized, written centered, and not exceeding 15 words. The English title must fully correspond to the Turkish title, written in 14 points and bold.

**Abstract:** Turkish and English abstracts should not exceed 300 words each. In Turkish and English abstracts, the words "Öz" and "Abstract" should be used, respectively. The abstract should include the purpose of the study, how it was done, the results and the author(s) comments on the results. References should not be used in the Abstract section.

**Keywords:** 1 line below the abstracts, the first letter of each keyword should be written in capital letters, the rest should be written in lowercase, if possible, maximum 6 keywords that are not used in the title and that best describe the work should be written.

**Introduction:** In this section; the subject of the study, its justification, previous studies directly related to the subject and the purpose of the study should be given.

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**Discussion:** Findings should be discussed with current manuscripts about the study, but unnecessary repetitions should be avoided. The similarities and differences of the findings with other studies should be given and the reasons should be explained.

**Acknowledgment:** You should list acknowledgments at the end of the text, in a separate section before references, and not include them on the title page, as a footnote in the headline, or otherwise. In this section, you can list the natural and legal persons who provide financial or moral assistance during the research.

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

**Declaration of Conflict of Interest:** Corresponding authors, on behalf of all the authors of a submission, must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

**References:** Citations and bibliography should be prepared with the Endnote program. Endnote-style files of our journal prepared in APA format are shared separately for Turkish and English manuscripts, with download links at the file upload step.

While writing the references list, the first author should be listed alphabetically according to the surname, the lines after the first line should start 1.0 cm from the right. Different works of the same author/authors should be listed starting from the old date, and works with the same date starting from a single author. References should be presented in their original language whenever possible. References that are not given in their original language can be given in Turkish or English. However, in this case, the original language of the source should be specified in parentheses.

• If the source is an article: Author's surname, initials., year, title of the article, name of the journal (italic), volume number (if any), page range.

Özgören, M., 2006, Flow Structure in the downstream of square and circular cylinders, Flow Measurement and Instrumentation, 17 (4), 225-235.

• If the source is a book: Author's surname, initial(s), year, name of the book, volume number, editor(s) / translation editors, if any, place of publication (italics), place of publication, page range.

Dasgupta, D., 1998, Artificial immune systems and their applications, Springer-Verlag, Berlin - Heidelnerg, 45-52.

Note: In translated books, the publication date of the translated book, not the original book, will be taken as a basis.

• If the source is the printed thesis: Author's surname, initial(s). (year), Title of the Thesis", Type of Thesis (Master/PhD), Graduate School of the Thesis (italics), place of submission, page range.

• If the source is taken from the congress: Author's surname, initial(s), year, Name of the Paper, name of the congress, seminar or conference (italic), place, page range in the proceedings book.

Güneş, S. and Polat, K., 2009, Medical diagnostic support system based on the use of least square support vector machines in electrocardiogram (ECG) arrhythmia diagnosis, 13th Biomedical Engineering National Meeting, BİYOMUT-2009, İstanbul, 170-173.

• If the source is taken from the report: Author's surname, initial(s) (if the report is a legal entity, the name of the organization), year, name of the report, short name of the organization that prepared the report and report number (italic), place of publication (italic), page range .

De Castro, L. N. and Von Zuben, F. J., 2000, Artificial immune systems: Part I- Basic theory and applications, DCA-RT 02/00, Brasil, 23-28.

• If the source is taken from the current magazine and newspaper news:

Corliss, R., 1993, Pacific Overtures Times, 142 (11), 68-70.

• If the source is from a national study whose author is unknown:

Anonymous, 2006, Agricultural statistics summary, DİE Publications, Number; 12, Ankara, 22-23.

• If the source is taken from a foreign work whose author is unknown:

Anonymous, 1989, Farm accountancy data network, an A-Z of methodology, Commission Report of the EC, Brussels, 16-19.

• If more than one publication of the same author published in the same year is used, an alphabetic character is added to the end of the year of publication. For example, for three publications by the same author(s) in 2003 (2003a, 2003b, 2003c).
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Author's surname, initial(s)., year, Title, Scale, Publication: Publisher.
Mason, J., 1832, Map of the countries lying between Spain and India, 1:8.000.000, London: Ordnance Survey.
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Author's surname, initial(s)., year, Title [online], (Edition), Place of Publication, Web address:URL [Date of Visit].
Holland, M., 2002, Guide to citing Internet sources [online], Poole, Bournemouth University,http://www.bournemouth.ac.uk/library/using/guide_to_citing_internet_sourc.html [Visit Date: November 4, 2002].
**Figures and Tables:** Figures, graphics, photographs and the like should be specified as "Figure" and numerical values as "Table". All figures and tables should be embedded in the manuscript. Figures and tables should be no more than 16x20 cm in length in single-page layout and 8 cm in width in double-column layout. The size of the figures and tables should be in a resolution that can be printed. Pictures supporting the research results should be in "jpg" format with 600 dpi resolution. Each table and figure should be cited in the text. All tables and figures should be numbered sequentially throughout the manuscript (Table 1 and Figure 1). Table and figure titles and descriptions should be short and concise. Headings for figures and tables should be in 10 points, texts in figures and tables should be in 9 points, texts under tables should be in 8 points Times New Roman font. If abbreviations are used in tables and figures, these abbreviations should be explained just below.
**Units:** SI (System International d'Units) measurement units should be used in all manuscripts. Use a dot as a decimal fraction (like 1.25 instead of 1,25). "/" should not be used in units and a space should be given between units (such as m s-1 instead of m/s, J s-1 instead of J/s, kg m s-2 instead of kg m/s2). A space must be left between the number and the symbol (such as 4 kg N ha-1, 3 kg m−1 s−2, 20 N m, 1000 s−1, 100 kPa, 22 °C). Exceptions to this rule are the degrees, minutes, and seconds symbols (°, ′, and ″) used for planar angles. They should be placed immediately after the number (like 10°, 45', 60"). The abbreviation of liter should be indicated as "l". If they are not at the end of the sentence, do not put a period at the end of the symbols (kg, not kg.).
**Formulas:** Formulas should be numbered and the formula number should be shown in parentheses, aligned to the right next to the formula. Word math processor should be used in writing the formulas, main characters should be in 12 points, variables should be in italics, numbers and mathematical expressions should be given plain. If it is to be cited in the text, it should be given in the form of "Equation 1" (…the related model is given in Equation 1).

# SELÇUK ÜNİVERSİTESİ FEN FAKÜLTESİ
# FEN DERGİSİ

# SELCUK UNİVERSITY JOURNAL OF SCIENCE FACULTY

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**İÇİNDEKİLER / CONTENTS**

*Araştırma Makaleleri / Research Articles*

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*Research Article*

# Analysis of Symptoms and Demographic Characteristics in Diagnosis of COVID-19 by Logistic Regression Model

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ABSTRACT

The new coronavirus COVID-19 is an infectious disease that started spreading globally in December 2019. Some symptoms are known to give clues as to whether the COVID-19 virus is infected. Therefore, the main purpose of this paper was to determine specific symptoms related to COVID-19 for the rapid diagnosis of COVID-19 cases. The data set consists of 25985 individuals including PCR results, 2 demographic properties (age, gender), and 5 symptoms such as headache, shortness of breath, sore throat, fever, and cough is considered in this study. We analyzed the relationship between these covariates and PCR results by binary logistic regression model. A total of 16405 (63.1%) individuals having to positive PCR results were included in this study. The research population was divided into two age groups (<60 and ≥60). The findings regarding the symptoms observed in COVID-19 patients can be listed as follows: Headache (25.8%), shortness of breath (2.2%), sore throat (11.2%), fever (16.3%), and cough (26.2%). The findings of binary logistic regression analysis show that any individual in the elder group has more probability of a positive PCR result approximately 1.6 times (odds ratio [OR]: 1.681), 95% confidence interval [CI]: 1.535-1.840). Also, an individual with symptoms of headache is approximately %7 more likely to have a positive PCR result than a nonexistent one (OR: 1.068, CI: 1.006-1.135).

*Araştırma Makalesi*

# COVID-19 Tanısında Semptomların ve Demografik Özelliklerin Lojistik Regresyon Modeli ile Analizi

MAKALE BİLGİSİ

*Makale Geçmişi*
Geliş 1 Ağustos 2023
Revizyon 17 Eylül 2023
Kabul 24 Ekim 2023

*Anahtar Kelimeler*
İkili lojistik regresyon
COVID-19
Bulaşıcı hastalık
Semptomlar
Odds oranı

ÖZ

Yeni koronavirüs COVID-19, Aralık 2019'da küresel olarak yayılmaya başlayan bulaşıcı bir hastalıktır. Bazı semptomların COVID-19 virüsünün enfekte olup olmadığına dair ipuçları verdiği bilinmektedir. Bu nedenle, bu makalenin temel amacı COVID-19 vakalarının hızlı teşhisi için COVID-19 ile ilgili spesifik semptomları belirlemektir. PCR sonuçları, 2 demografik özellik (yaş, cinsiyet) ve baş ağrısı, nefes darlığı, boğaz ağrısı, ateş ve öksürük gibi 5 semptomu içeren 25985 kişiden oluşan veri seti bu çalışmada dikkate alınmıştır. Bu ortak değişkenler ile PCR sonuçları arasındaki ilişki ikili lojistik regresyon modeli ile analiz edilmiştir. PCR sonucu pozitif olan toplam 16405 (%63,1) birey bu çalışmaya dahil edilmiştir. Araştırma popülasyonu iki yaş grubuna ayrılmıştır (<60 ve ≥60). COVID-19 hastalarında gözlenen semptomlara ilişkin bulgular şu şekilde sıralanabilir: Baş ağrısı (%25,8), nefes darlığı (%2,2), boğaz ağrısı (%11,2), ateş (%16,3) ve öksürük (%26,2). İkili lojistik regresyon analizi bulgularına göre, yaşlı gruptaki herhangi bir bireyin pozitif PCR sonucu alma olasılığı yaklaşık 1,6 kat daha fazladır (odds oranı [OR]: 1,681), %95 güven aralığı [CI]: 1.535-1.840). Ayrıca, baş ağrısı semptomları olan bir bireyin pozitif PCR sonucuna sahip olma olasılığı olmayanlara göre yaklaşık %7 daha fazladır (OR: 1.068, CI: 1.006-1.135).

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Yazar, anonimleştirilmiş Kovid-19 hasta kayıtlarına kamu erişimi sağladığı için İsrail Sağlık Bakanlığı'na ve Kovid-19 hastalarının verilerinin elde edilmesindeki yardımlarından dolayı Prof. Dr. David Gurwitz'e müteşekkirdir. The author is thankful to the Israel Ministry of Health for providing public access to anonymized Covid-19 patient records and to Prof. Dr. David Gurwitz for his help in obtaining data of Covid-19 patients.

## 1. Introduction

The new coronavirus (COVID-19) appeared in Wuhan, China in December 2019 and rapidly exceeded its borders and affected the whole world. The World Health Organization (WHO) officially reported that COVID-19 is a global pandemic on March 11, 2020 (WHO, 2020). As of February 21, 2023, it was globally announced that there have been 757,264,511 confirmed COVID-19 cases with 6,850,594 deaths by the WHO (WHO, 2023). The rapid spread of COVID-19 has devastated the health system even in developed countries. Rapid investigation and identification of epidemiologic features of new infectious diseases are critical for reducing transmission and effective treatment management (Ki, 2020). Early diagnosis of the disease is very important to combat this epidemic affecting the world. In this regard, some clinical symptoms have helped in the early diagnosis of COVID-19. The main symptoms of COVID- 19, such as cough, fever, and dyspnea are important in the early diagnosis of COVID-19 disease (Alimohamadi et al., 2020). Identifying the clinical symptoms of COVID-19 in children is crucial in terms of accurately predicting the course of the disease (Tang et al., 2021). Many scientific studies have been conducted to specify the role of these symptoms in the diagnosis of COVID-19. (Ki, 2020) examined the epidemiologic properties of early COVID-19 cases in Korea. Choi and Ki (2020) designed a mathematical model to determine the effects of social distancing on the spread of COVID-19 in Korea. Yang et al. (2020) provided a systematic analysis of the prevalence of some symptoms in COVID-19 patients. Zhou et al. (2020) estimate the fatality of COVID-19 patients via a logistic regression model. Guan et al. (2020) examined the clinical characteristics of 1,099 COVID-19 patients in China. In another Chinese research, Xu et al. (2020) provided clinical findings of characteristics in COVID-19 patients. Feng et al. (2020) studied the early prediction of illness progression in COVID-19 patients with some clinical characteristics. Yupari-Azabache et al. (2021) analyzed the mortality risk factors in COVID-19 patients by using the logistic regression model. Hills and Eraso (2021) discussed the reasons for non-adherence the social distancing during the COVID-19 pandemic using a logistic regression analysis. Sonoda et al. (2021) focused on the determination of common symptoms in COVID-19 patients from Japan. Xiong et al. (2020)studied on the estimation of the fatality rates of COVID-19 patients from California using a logistic regression. In this regard, they considered some factors such as gender, race, and age to estimate the fatality rates of COVID-19 patients. Fleitas et al. (2020) analyzed the role of clinical symptoms in COVID-19 patients from Argentina via a logistic regression model. They examined the roles of 23 different symptoms in discrimination of being with COVID-19 positive. Liu et al. (2022) analyzed the clinical properties and risk factors in COVID-19 patients from Wuhan, China by using the logistic regression model.

## 2. Materials and Methods

The research population consisted of 25685 individuals with 16405 (63.1%) COVID-19 positive and 9580 (36.9%) COVID-19 negative. The research data is open-access data released to the public by the Israel Ministry of Health (Health, 2021). This anonymized dataset was constructed on July 29, 2020. This data is constantly updated. The last update date is March 7, 2021 (Health, 2021) for the used data set in this paper. These data were collected December 11-31 2020 in Israel. The Binary logistic regression model was designed to determine which symptoms or demographic variables affect the being COVID-19 positive. In this regard, the dependent variable is considered PCR result (negative = 0, positive = 1) and gender, age, cough, fever, shortness of breath, and sore throat are chosen as independent covariates in the binary logistic regression model. A two-sided α of less than 0.05 was considered statistically significant. The demographic findings were expressed as frequency (%).

## 3. Results and Discussion

The epidemiological characteristics of a total of 25685 individuals in this research are presented in Table 1. It is seen that the < 60 group ($n$ = 23347(89.8%)) and the ≥ 60 group ($n$ = 2638(10.2%)) are presented.

**Table 1.** The descriptive statistics of covariates in regression models.

| Covariate | Category | Frequency (%) |
|---|---|---|
| PCR result (Response) | Positive | 16405 (63.1) |
| | Negative | 9580 (36.9) |
| Gender | Male | 11889 (45.8) |
| | Female | 14096 (54.2) |
| Age | <60 | 23347 (89.8) |
| ≥ 60 2638 (10.2) | | |
| Headache | Absent | 19398 (74.7) |
| | Present | 6587 (25.3) |
| Shortness of breath | Absent | 25449 (97.9) |
| | Present | 536 (2.1) |
| Sore throat | Absent | 23146 (89.1) |
| | Present | 2839 (10.9) |
| Fever | Absent | 21698 (83.5) |
| | Present | 4287 (16.5) |
| Cough | Absent | 19321 (74.4) |
| | Present | 6664 (25.6) |

Table 2 provides the results of the univariate logistic regression model. Age, headache, shortness of breath, sore throat, and cough were included in the multivariate analysis, as they were found to be statistically significant ($p$ < 0.05) in Table 2.

**Table 2.** The results of the univariate logistic regression model.

| | $\hat{\beta}$ | SE $\left(\hat{\beta}\right)$ | p-value | OR | 95 % OR |
|---|---|---|---|---|---|
| Gender | 0.021 | 0.026 | 0.411 | 1.021 | 0.971-1.074 |
| Age | 0.518 | 0.046 | <0.001* | 1.678 | 1.533-1.836 |
| Headache | 0.068 | 0.03 | 0.022* | 1.07 | 1.01-1.135 |
| Shortness of breath | 0.225 | 0.094 | 0.016* | 1.252 | 1.042-1.504 |
| Sore throat | 0.085 | 0.042 | 0.041* | 1.089 | 1.004-1.181 |
| Fever | -0.046 | 0.035 | 0.182 | 0.955 | 0.893-1.022 |
| Cough | 0.085 | 0.03 | 0.004* | 1.089 | 1.028-1.154 |

Table 3 provides the results of the multivariate logistic regression model. According to Table 2, it is seen that age and headaches are significant in the multivariate logistic regression model ($p$ < 0.05). Those older than 60 years of age were 1.681 times more likely to be PCR positive than those younger than 60 years old (OR: 1.681, [1.535−1.840]). Those with headache symptoms are 1.068 times more likely to be PCR positive than those without headache symptoms (OR:

1.068[1.006−1.135]). In other words, older age and the presence of headache are statistically significant in determining the PCR test result and are variables that increase the probability of being COVID-19 positive.

**Table 3.** The results of the multivariate logistic regression model.

| | $\hat{\beta}$ | SE $(\hat{\beta})$ | p-value | OR | 95 % OR |
|---|---|---|---|---|---|
| Age | | | | | |
| <60 (Ref) | | | | | |
| >60 | 0.519 | 0.046 | <0.001* | 1.681 | 1.535-1.840 |
| Headache | | | | | |
| Absent (Ref) | | | | | |
| Present | 0.066 | 0.031 | 0.032* | 1.068 | 1.006-1.135 |
| Shortness of breath | | | | | |
| Absent (Ref) | | | | | |
| Present | 0.166 | 0.095 | 0.080 | 1.180 | 0.980-1.421 |
| Sore throat | | | | | |
| Absent (Ref) | | | | | |
| Present | 0.062 | 0.043 | 0.153 | 1.064 | 0.977-1.157 |
| Cough | | | | | |
| Absent (Ref) | | | | | |
| Present | 0.039 | 0.031 | 0.207 | 1.039 | 0.979-1.104 |
| Constant | 0.453 | 0.017 | <0.001* | 1.572 | |

*: It means statistical significance ($p$<0.05).

**Table 4.** Results of goodness of fit test for the multivariate logistic regression model.

| Tests of goodness of fit | Test | df | P |
|---|---|---|---|
| Omnibus Tests of Model Coefficients | 151.038 | 5 | <0.001 |
| Hosmer and Lemeshow Test | 4.085 | 3 | 0.252 |

From Table 4, the results of the Hosmer-Lemeshow test show that the logistic regression model fits the data ($p$=0.252>0.05). According to omnibus tests of model coefficients, the coefficients of covariates are statistically significant in the multivariate logistic regression model, and the model formed by the explanatory variables is statistically significant ($p$<0.05).

This study is designed to determine which symptoms or characteristic properties have an impact on positive PCR results. Considering the findings of previous studies for similar purposes, the results of our study are similar to the results of many previous studies. Yang et al. (2020) reported the most observed clinical symptoms can be listed as follows: fever (91.3%), cough (67.7%), fatigue (51%), and dyspnea (30.4%). It is observed that the most common two symptoms are fever and cough in (Alimohamadi et al., 2020; Guan et al., 2020; Liu et al., 2022). [16] found that headache (OR:1.71) significantly correlated with being COVID-19 positive in the investigation including 48748 individuals less than 56 years old. Thus, they determined that the headache is a factor that increases the probability of being COVID-19 positive. Fleitas et al. (2020) observed that although the most common symptoms are cough and fever, there is no correlation between these symptoms and being COVID-19 positive. It is reported that older age is significant for COVID-19 disease severity while fever and cough are not significant in determining COVID-19 disease severity(Fleitas et al., 2020; Yupari-Azabache et al., 2021). The findings of Sonoda et al. (2021) showed that headache (OR: 3.31) is significantly associated with being COVID-19 positive. They mentioned that the presence of fever was not utility to determine being COVID-19 positive. Guan et al. (2020) observed that some characteristics such as gender, fever, cough, sore throat, and shortness of breath are not significant to determine COVID-19 positive while age groups are crucial in the diagnosis of COVID-19. It is emphasized that age is an important factor in the risk of death due to COVID-19 (Fleitas et al., 2020; Xiong et al., 2020). Yupari-Azabache et al. (2021) mentioned that an older person is a risk factor mortality of hospitalized patients infected with COVID -19 (OR: 1.11). The findings of Feng et al. (2020), some characteristics such as gender, fever, cough, and shortness of breath are not statistically significant to estimate of illness progression in COVID-19 patients. According to our findings, the most common symptoms are cough and headache in COVID-19 patients. Headache (OR: 1.681) and age (OR: 1.068) were found statistically significant in the multivariate logistic regression model while gender, cough, fever, shortness of breath, and sore throat were found not significant in determining the PCR results.

## 4. Conclusions

This study includes many results that we think will contribute to the literature. First of all, headache and age were found to be significant in the multivariate logistic regression analysis, and it was concluded that these two variables were effective in determining the PCR test result. The presence of a headache increases the probability of being COVID-19 positive by 1.068 times, while someone ≥60 age is 1.681 times more likely to be COVID-19 positive than someone <60 age. As a result, we provide new important findings of some symptoms and characteristics in the literature. The conclusions of this research have supported previous findings. We consider that the results of this study will guide future studies. One of the important advantages of this study is that it was designed with a big sample size (n = 25985). The study has limitations. Firstly, the Covid-19 data sets are anonymous and open Access (Health, 2021). Secondly, the sample size is limited and an accurate assessment of the diagnosis of Covid-19 patients is not accurate.

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*Research Article*

# Preparation of Prebiotic Pectin-Supplemented Vitamin C Microcapsules

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ABSTRACT

Microencapsulating vitamin C with dietary fibers and prebiotics can improve the storage, preservation, and marketing of vitamin C supplements. This research aimed to explore the feasibility of creating microcapsules using vitamin C, pectin, and alginate through a microencapsulation technique. Pectin was extracted from lemon peel using an acid treatment and then characterised. The morphology of the vitamin C-pectin-alginate microcapsules was examined by scanning electron microscopy. Time, temperature, and pH-dependent vitamin C release profiles of the vitamin C-pectin-alginate microcapsules were studied. The rate of release of vitamin C increased towards pH values close to 7.0, with a higher rate of 83.97% observed at pH 7.0. Additionally, temperature affected the release of vitamin C from the microcapsules, with approximately 47.2% release at body temperature (37°C) and a higher fluctuation in vitamin C release was observed at 20°C. This study revealed that pectin extracted from lemon peels can be used with alginate to encapsulate vitamin C.

*Araştırma Makalesi*

# Prebiyotik Pektin Takviyeli C Vitamini Mikrokapsüllerinin Hazırlanması

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ÖZ

C vitamininin diyet lifleri ve prebiyotiklerle mikrokapsüllenmesi, C vitamini takviyelerinin depolanmasını, korunmasını ve pazarlanmasını iyileştirebilir. Bu çalışmanın amacı, bir mikrokapsülleme tekniği ile C vitamini, pektin ve aljinat kullanarak mikrokapsüller oluşturmanın fizibilitesini araştırmaktır. Pektin, asit muamelesi kullanılarak limon kabuğundan ekstrakte edildi ve karakterize edildi. C vitamini-pektin-aljinat mikrokapsüllerinin morfolojisi taramalı elektron mikroskobu ile incelenmiştir. C vitamini-pektin-aljinat mikrokapsüllerinin zaman, sıcaklık ve pH bağımlı C vitamini salım profilleri incelenmiştir. C vitamini salım hızı, pH 7.0'a yakın pH değerlerine doğru artarken, pH 7.0'da daha yüksek oranda %83.97 gözlendi. Ek olarak sıcaklık, vücut sıcaklığında (37°C) yaklaşık %47.2 salınım ile mikrokapsüllerden C vitamini salınımını etkiledi ve 20°C'de C vitamini salınımında daha yüksek bir dalgalanma gözlendi. Bu çalışma, limon kabuklarından ekstrakte edilen pektinin, C vitaminini kapsüllemek için aljinat ile birlikte kullanılabileceğini ortaya koydu.

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## 1. Introduction

Vitamin C is a water-soluble vitamin with antioxidant properties found in fruits and vegetables. It prevents cellular damage and lowers the risk of cancer by neutralizing reactive oxygen species that cause DNA mutations (Sdiri et al., 2020).

Vitamin C acts as a cofactor for collagen synthesis, balances hormones, and aids in cellular metabolism (Skrovankova et al., 2015). It can protect against oxidative damage by binding to metal ions or scavenging reactive oxygen species (Ngai et al., 2013; Yuswan et al., 2015). As a reducing agent, the ascorbic acid molecule is sensitive to oxidation. Its exposure to air, moisture, and heat causes it to oxidise, resulting in the loss of its bioactivity in its oxidised form (Zielinski et al., 2001; Shimoni, 2004; Blažević et al., 2020).

On average, women should consume 75mg and men 90mg of vitamin C daily (Blažević et al., 2020). To preserve the bioactivity of ascorbic acid and prevent oxidation, different encapsulation methods have been developed for its controlled release in pharmaceutical applications (Cho et al., 2003; Champagne and Fustier, 2007). For example, in one study, the stability of chitosan nanoparticles with L-ascorbic acid was examined during heat treatment in aqueous solutions (Jang and Lee, 2008). In another study, spray-dried microspheres with cross-linked chitosan and tripolyphosphate enabled the release of encapsulated ascorbic acid (Desai and Park, 2006).

Pectin (a.k.a. pectic polysaccharides) is a complex and heterogeneous polysaccharide found in plant cell walls, made of galacturonic acid and its methyl ester (Fishman et al., 1999). Pectin is found in dicotyledonous plants and plays a structural role. It's commonly associated with cellulose and hemicellulose and can be found in citrus fruits, apples, and sugar beets (Santos et al., 2020). Oranges and limes have pectins in their albedo (white part on the inside of the peel), mostly made up of anhydrogalacturonate. Homogalacturonans may also contain some xylose units. Rhamnogalacturonan is the second most common component, with side chains of arabinan, galactan, and arabinogalactan (Fishman et al., 2003).

Pectin is a versatile substance extracted from fruit peels. It's used in food and pharmaceuticals as a gelling, thickening, and emulsifying agent. Thousands of tons of citrus fruit peels are processed annually to obtain pectin, which is essential for human health and nutrition. Pectic polysaccharides, found in dietary fibres, can help regulate lipid metabolism (Groudeva et al., 1997) and decrease glucose absorption in those with diabetes (Schwartz et al., 1988).

Pectic polysaccharides can stop pathogens from attaching to the intestinal mucosa. Moreover, they can be fermented by probiotic bacteria into short-chain fatty acids that can prevent colon necrosis (Wang and Friedman, 1998; Jun et al., 2006). Furthermore, certain types of pectin possess immunomodulatory properties that can impact the gastric mucosal immune system by activating Peyer's patch cells, leading to increased proliferation of lymphocytes and macrophages (Yamada and Kiyohara, 2007; Kratchanova et al., 2010). Earlier reports suggest that citrus pectins and their modified derivatives may have a preventative impact on the growth and spread of cancer (Ramachandran et al., 2011). Recent studies have indicated that pectins extracted from citrus fruits can be considered a viable prebiotic, supplying dietary fibre to probiotic bacteria in the gut microbiome (Ho et al., 2017; Islamova et al., 2017; Zhang et al., 2018).

Alginate is a naturally occurring polysaccharide commonly found in marine brown algae (Phaeophyceae). It is a structural component in soil bacteria, serving as a capsular polysaccharide (Robyt, 1998). Alginates are versatile additives used in food (E401), medicine, pharmaceutical, and textile industries for their ability to gel, thicken, stabilize, and retain water. Alginates undergo rapid crosslinking and sol/gel transition with $Ca^{2+}$ ions, creating adjustable gels with temperature-insensitive properties in water-based solutions (Steinbüchel and Rhee, 2005; Donati et al., 2009). Additionally, alginate can retain fluids, making it useful for drug delivery, tissue engineering, gene delivery (Josef et al., 2010), and regenerative therapy (Smidsrød and Skja, 1990). Alginates are bioadhesive and can target mucosal tissues. They can also effectively encapsulate substances using microencapsulation, which involves coating particles with a polymer film (Bitton et al., 2006).

As discussed above, though there have been some formulations for vitamin C supplements in the literature, little attention has been paid to vitamin C-alginate-pectin microcapsules. Encapsulating vitamin C molecules in pectin and alginate may create a protective barrier against oxygen molecules that can cause the loss of biological activity of ascorbic acid. This can improve the storage, preservation, and marketability of vitamin C dietary supplements. Combining the pectin and vitamin C extracted from lemon peel can produce a prebiotic nutritional fibre source essential for the probiotic bacteria in the gut flora. This research explored the feasibility of producing microcapsules containing vitamin C using pectin isolated from lemon peels and alginate, a safe and edible polysaccharide known for its biocompatibility and mucoadhesive properties.

## 2. Experimental

### *2.1. Materials and method*

#### *2.1.1. Extracting pectin from lemon peels*

A method reported in the literature was followed to extract pectin (Azad et al., 2014). Lemon peels (*Molla Mehmet*) were obtained from a local market and used for pectin extraction. To extract the lemon peel's albedo (white inner layer), a knife was used to remove it, followed by drying at room temperature (20°C). The dried samples were then placed in a citric acid solution (5.0 g in 100 mL of distilled water), and an HCl solution was added until the pH reached 1.0. The solution was stirred at 80°C for an hour before cooling to room temperature (20°C) and filtering through paper. The filtrate was cooled to +4°C and allowed to gel with added ethanol. 100 mL of 96% ethanol was added to the filtrate and kept at 4°C for 12 hours, allowing the pectin to precipitate. After the precipitated pectin was isolated by filtration, it was washed with ethanol. The gelled sample was dried in a Petri dish at room temperature (20°C). To reduce moisture content, the dried sample was kept in an oven at 60°C for an hour and then crushed into powder using a mortar.

The structural analysis of the pectin samples was done by FT-IR spectroscopy (Bruker Vertex 70 FT-IR spectrometer, 4000-400 $cm^{-1}$). The extraction yield, equivalent weight, moisture, ash, and methoxyl content of pectin were determined using a previously published method (Azad et al., 2014).

***Pectin yield*:** Pectin yield was calculated by averaging at least 3 replicates using Equation 1. The pectin samples were stored in glass bottles at 4°C.

$$\% \text{ Pectin Yield} = \left(\frac{\text{mass of pectin}}{\text{mass of albedo}}\right) \text{x } 100 \quad \text{(Eq. 1)}$$

***Moisture and ash content*:** To determine the moisture content of lemon peels dried at room temperature (20°C), the following steps were taken: 1.0 g of dried fruit peel ($W_1$) was placed in a weighted Petri dish (W), then put in a 100°C oven for 5 hours. After cooling it in a desiccator, the dish was weighed again ($W_2$). The per cent moisture content of lemon peels was calculated by Equation 2.

$$\% \text{ Moisture} = \left(\frac{W1 - W2}{W1 - W}\right) \text{x } 100 \quad \text{(Eq. 2)}$$

$W_1$: mass of dried fruit peel, g
$W_2$: mass of Petri dish + sample, g
W: mass of Petri dish, g

To find out the ash content of the lemon peels (free from moisture), the sample was placed in a Petri dish and kept in a furnace at 600°C for 6 hours. After cooling to room temperature (20°C) in a desiccator, the dish's final weight (W3) was recorded. Using Equation 3, the percentage of ash content in the lemon peels was then calculated.

$$\% \text{ Ash} = \left(\frac{W3 - W1}{W2 - W1}\right) \text{x } 100 \quad \text{(Eq. 3)}$$

$W_3$: final mass of Petri dish, g
$W_1$: mass of Petri dish, g
$W_2$: mass of Petri dish + sample amount, g

***Equivalent weight*:** In a 250 mL flask, 0.5 g of pectin sample was taken and mixed with 5.0 mL of ethanol. Then, 1.0 g of sodium chloride and 100 mL of distilled water were added to the mixture. To prepare phenol red, 0.077 g of it was dissolved in 100 mL of water. Afterwards, 6 drops of the prepared phenol red were added to the solution and titrated against 0.1 M NaOH. At the titration point, a colour transformation close to purple was observed. This neutralised solution was stored for the determination of methoxyl content. The calculation of the equivalent mass was performed using Equation 4.

$$\% \text{ Equivalent weight} = \left(\frac{\text{Sample mass x } 1000}{\text{Alkaline mL x Alkaline molarity}}\right) \quad \text{(Eq. 4)}$$

***Methoxyl content*:** To determine the methoxyl content, the stored sample from the equivalent mass determination was taken, and 25 mL of sodium hydroxide (0.25 M) was added to the sample taken. The mixed solution was thoroughly mixed and incubated at room temperature (20°C) for 30 minutes. After 30 minutes, 25 mL of 0.25 M hydrochloric acid was added and titrated against 0.1 M NaOH. The methoxyl content was calculated according to Equation 5 below:

$$\% \text{ Methoxyl content} = \left(\frac{\text{Alkaline mL x Alkaline molarity x } 31}{\text{Sample mass}}\right) \quad \text{(Eq. 5)}$$

*2.1.2. Encapsulation of vitamin C*

Sodium alginate (5.0 g) was stirred at 1000 rpm for 30 minutes at room temperature (20°C) to dissolve in distilled water (100 mL). After adding vitamin C (2.0 g) and pectin (0.5 g) to the mixture, it was mixed at 3500 rpm for 30 minutes to ensure homogeneity. Mixing was carried out in a closed vessel to reduce contact with air during mixing. Afterwards, the mixture was stirred at 750 rpm for 15 minutes to prevent bubble formation. The mixture was transferred to a burette, and a microcapsule was formed by dripping into a $CaCl_2$ solution (5.0 g in 100 mL of distilled water). The microcapsules in the calcium chloride solution were removed by filtration and washed with distilled water. It was stored in glass Petri dishes at +4°C without exposure to sunlight. The amount of ascorbic acid molecules encapsulated in the microcapsules was calculated spectrophotometrically by measuring their absorbance at 260 nm (using a calibration curve based on absorbance measurements).

To preserve the vitamin C microcapsules, they were dried at room temperature (20°C) in a dark environment and then stored in amber glass bottles at 4°C to avoid exposure to sunlight. The size, shape, and surface properties of the dried microcapsules were analysed using Scanning Electron Microscopy (SEM).

*2.1.3. Release characteristics of vitamin C microcapsules*

The release of ascorbic acid from vitamin C microcapsules was studied under the conditions listed below:

The release of ascorbic acid from vitamin C microcapsules was investigated under the following conditions.

Parameters to be tested:

Duration: 0.25 – 0.50 – 1 – 2 – 4 – 8 – 16 – 24 hours (pH: 7.35, 37°C)

Temperature: 20 – 25 – 30 – 37°C (pH: 7.35, 1 hour)

pH: 2.0 – 3.0 – 4.0 – 5.0 – 6.0 – 7.0 – 8.0 (1 hour, 37°C)

In a typical experiment, 100 mg of vitamin C microcapsules were placed into 50 mL of distilled water and agitated at 50 rpm at a specified temperature, 2.0 mL of solution was taken at regular intervals. Its absorbance was measured at 260 nm in a UV-vis spectrophotometer, the amount of ascorbic acid in the solution was determined with the help of the calibration curve. Fresh ascorbic acid solutions were prepared and diluted to a certain extent for the calibration plot drawing. Dilute solutions of HCl and NaOH were used to adjust the pH.

## 3. Results and Discussion

### *3.1. Extraction of pectin from lemon peels*

In the study, the pectin yield obtained from lemon albedo (the white part on the inside of the peel) was found to be 60.88%. The pectin content was higher than a report in the literature, which was around 13% (Azad et al., 2014). In our study, a specific type of lemon (*Molla Mehmet*) was chosen because of its thick skin and plentiful albedo. The fibrous inner portion of the fresh peel (albedo), which contains a high amount of pectin, was removed and used in the extraction. As a result, the extraction procedure followed in the study yielded a notably high pectin extraction yield.

Pectin is a significant ingredient in the food industry, and it is extracted from entire citrus peels for industrial purposes (Pereira et al., 2016; Adetunji et al., 2017). Therefore, in the study, pectin was also extracted from whole lemon peels (dried and pulverised) using the same extraction procedure employed for lemon albedo to investigate what the pectin content of the lemon peel would be. The pectin yield obtained from the whole lemon peel was 27.06%, lower than in the previous study (30-35%) (Da Silva and Rao, 2006).

### *3.2. Characterisation of pectin from lemon peels*

The FT-IR spectrum of pectin obtained from lemon peels was obtained (Fig. 1) and compared with the commercial pectin spectrum (Baum et al., 2017; Şen et al., 2021). The spectrum has O-H stretching band at 3334 $cm^{-1}$ and the C-H stretch of the alkyl groups (CH, $CH_2$, $CH_3$) of galacturonic acid at 2932 $cm^{-1}$. The bands around 1730 $cm^{-1}$ and 1650-1510 $cm^{-1}$ and at 1733-1619 $cm^{-1}$ correspond to the C=O stretching vibration of esterified carbonyl groups and C=O of free carbonyl groups, respectively. As a result, the FT-IR spectrum analysis confirms that the obtained polysaccharides are pectin since the extracted lemon pectin sample spectrum has absorption bands compatible with the commercial pectin spectrum.

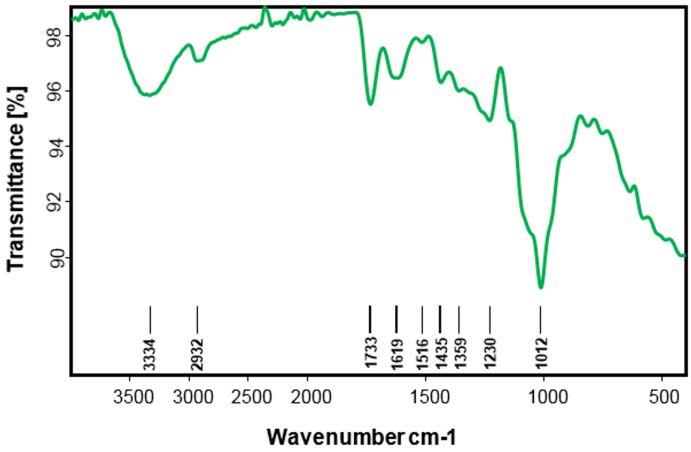


**Fig. 1.** FT-IR spectrum of pectin from lemon peel.

The moisture and the ash content of the pectin samples (previously dried at room temperature, 20°C) were found to be 10.13% and 0.43%, respectively, which was low and close to the figures (pectin: around 11% and ash: around 2-3%) reported in previous publications on pectin extraction from lemon, dragon fruit and sunflower head residues (Mohamadzadeh et al., 2010; Ismail et al., 2012; Azad et al., 2014). This low moisture content for pectin sources is desirable because excessive moisture levels in lemon peels can increase the proliferation of microorganisms and the secretion of pectinase enzymes, which can negatively impact the quality of the pectin (Maran et al., 2013). The lower ash content for pectin samples indicates their purity.

The equivalent weight value of extracted pectin depends on various factors, including the extraction method, the type of raw material used, the type of acid used for extraction, the extraction temperature, and the duration of extraction. The equivalent weight value of the extracted pectin was 6676.67 g. This high equivalent weight value indicates that the pectin samples were not undergone degradation during the extraction process (Ling et al., 2022). The methoxyl content of the pectin samples was found to be 4.96%, suggesting that the pectin extracted in the study could be classified as low-methoxyl pectin. Low-methoxyl pectins can form gels with low molecular mass sugars or divalent cations (Ismail et al., 2012). The methoxyl content is a crucial molecular index that determines pectin's ability to form a gel. This is because pectin's water solubility depends on the number and distribution of methoxyl groups and the degree of polymerisation (Peng et al., 2020).

### *3.3. Encapsulation of vitamin C with pectin and alginate*

The optimum parameters of microencapsulation were determined as follows: The microcapsule solution; the amount of sodium alginate: 5.0 g, the amount of ascorbic acid: 2.0 g, and the amount of pectin: 0.5 g in 100 mL distilled water and the gelation solution; the amount of CaCl2: 5.0 g in 100 mL distilled water. The encapsulation of vitamin C was performed under optimum parameters. The SEM images of the microcapsules are presented in Fig. 2. In the SEM images, the diameters of the microcapsules were determined to be approximately 1.8 mm (the upper left image). In addition, $CaCl_2$ crystal formations were observed on the surfaces of the microcapsules (the lower right image).

The study examined the release of vitamin C from the microcapsules under varying conditions of time, temperature, and pH. The outcomes are presented in Table 1. When the vitamin C microcapsules were exposed to an aquatic environment at a pH of 7.30 for over 8 hours, swelling of the microcapsules occurred, as shown in Fig. 3.

Additionally, it was observed that the release of vitamin C fluctuated over an 8-hour period, possibly due to the oxidation of ascorbic acid molecules (Njus et al., 2020). The partial dissolution of the alginate matrix in the capsules could have caused these deviations. However, the vitamin C microcapsules prepared in the study could remain structurally stable for up to 8 hours at a pH close to physiological pH and could release vitamin C. The rate of vitamin C release in 8 hours reached 99%.

**Table 1.** Time, temperature, and pH dependant vitamin C (AA: ascorbic acid) release from the vitamin C-pectin-alginate microcapsules.

| Time (h) | Amount of AA released (mg/mL) | Amount of AA released (%) | Temperature (°C) | Amount of AA released (mg/mL) | Amount of AA released (%) | pH | Amount of AA released (mg/mL) | Amount of AA released (%) |
|---|---|---|---|---|---|---|---|---|
| 0.25 | 19.13 | 57.16 | 20 | 20.88 | 62.37 | 2.0 | 10.85 | 32.42 |
| 0.50 | 20.65 | 61.68 | 25 | 5.60 | 16.73 | 3.0 | 15.75 | 47.06 |
| 1 | 26.13 | 78.07 | 30 | 4.79 | 14.26 | 4.0 | 21.93 | 65.51 |
| 2 | 26.83 | 80.15 | 37 | 14.11 | 47.16 | 5.0 | 14.81 | 44.25 |
| 4 | 28.93 | 86.42 | | | | 6.0 | 31.49 | 94.08 |
| 8 | 33.36 | 99.66 | | | | 7.0 | 28.11 | 83.97 |
| 16 | 20.53 | 61.32 | | | | 8.0 | 11.55 | 34.51 |
| 24 | 22.86 | 68.26 | | | | | | |

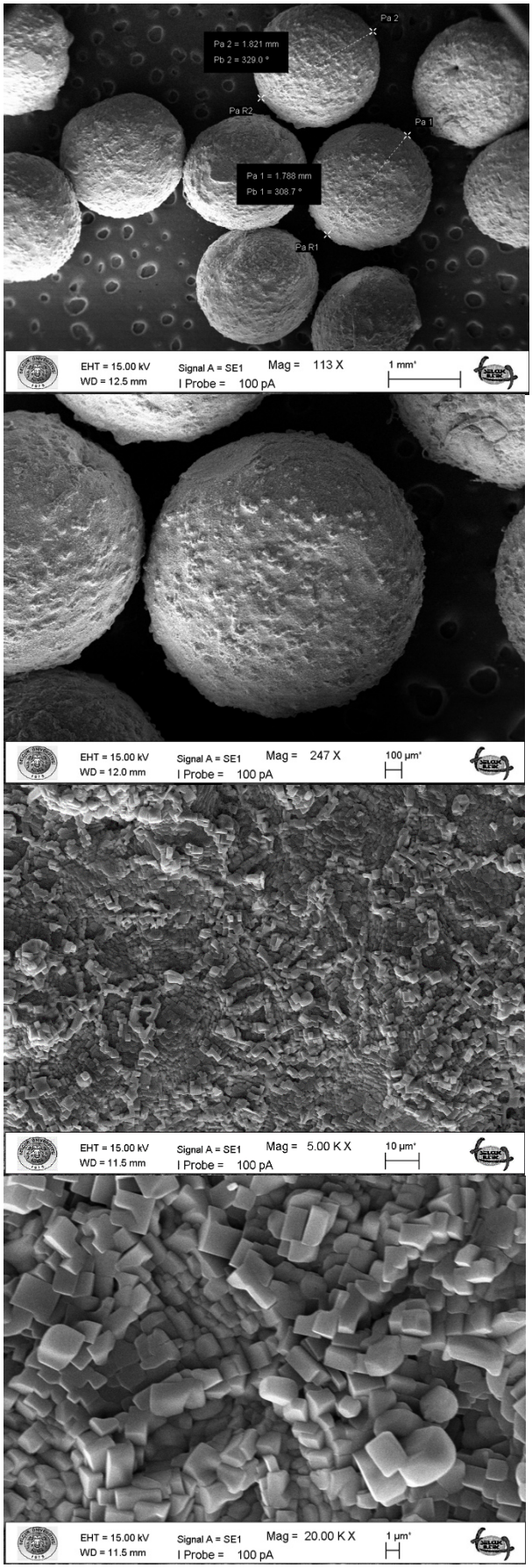


**Fig.2.** SEM images of vitamin C-pectin-alginate microcapsules at different magnifications.

**Fig.3.** Swelling was observed when the vitamin C microcapsules were kept at pH: 7.35 in an aquatic environment for 8 hours (in the right beaker).

The study showed that the microcapsules used for vitamin C encapsulation are suitable for absorption in the small intestine. The release rate of vitamin C increased towards pH values close to 7.0, with a higher rate of 83.97% observed at pH 7.0. This is significant as vitamin C absorption takes place in the small intestine (Said, 2011). Additionally, temperature affected the release of vitamin C from the microcapsules, with approximately 47.2% release at body temperature (37°C) and a higher fluctuation in vitamin C release was observed at 20°C.

Vitamin C promotes the growth and repair of skin and connective tissues (Li et al., 2018). In a previous study, a pectin/modified alginate buccal patch was proposed as a drug delivery device for treating oral cavity diseases (Özkahraman et al., 2023). To improve its mucoadhesive properties, alginate was modified with acrylic acid and thiolated with cysteine. The study examined the impact of vitamin C on the healing process of the buccal adhesive patch formulation. The results showed that vitamin C promoted fibroblast proliferation, migration, and collagen synthesis, which had a positive effect on the wound healing process, particularly during the epithelialisation phase.

## 4. Conclusions

This study revealed that pectin extracted from lemon peels can be used with alginate to encapsulate vitamin C. The research study demonstrated that the microcapsules utilized for vitamin C encapsulation are well-suited for absorption in the small intestine. The release rate of vitamin C increased as the pH values approached 7.0, with an 83.97% higher rate being observed at pH 7.0. The temperature had an impact on the release of vitamin C from the microcapsules, with about 47.2% of the release taking place at the average body temperature (37°C). There was a greater fluctuation in the release of vitamin C at 20°C. The findings demonstrated that the effects of temperature and time on microcapsule stability and vitamin C release should be addressed in further studies. This study is a preliminary study, the encapsulation of vitamin C with vegetable, animal, and bacterial polymeric carbohydrates should be investigated in future studies.

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**CRediT author statement**

ODO: Investigation, benchwork, study design, collection, analysis, and interpretation of data, manuscript writing, and funding acquisition.
SE: Investigation, benchwork, study design, collection, analysis, and interpretation of data, manuscript writing.
NO: Benchwork, experimental design, data collection, and manuscript writing.
IS: Conceptualization, study design, analysis and interpretation of data, manuscript writing, and supervision of the work.
GA: Conceptualization, experimental design, reviewing, and editing.

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*Research Article*

# Evaluation of Aflatoxin B1 Binding Capacity with Mix Toxin Binder using Central Composite Design

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ABSTRACT

Aflatoxin B1 (afl B1) binding capacity of a mixed toxin binder used in poultry nutrition were determined using the central composite design technique. Experimental conditions such as pH, temperature and incubation time for the determination of the binding capacity of a mix binder were optimized for Central Composite Design. The impact of these three independent variables on the % binding of aflatoxin B1 was evaluated at different five levels (-1.68, -1, 0, 1, 1.68). The optimum experimental conditions were 5.8, 42°C, 94.11 min for pH, temperature and incubation time, respectively using quadratic model and desirability function. A significant effect of each independent variable was observed on the % binding efficiency of aflatoxin B1. In optimum experimental conditions, aflatoxin B1 binding capacity with mix toxin binder was found 97%. The results of the present study indicated that the mix binder is very suitable for binding of aflatoxin B1 and the central composite design can be used effectively in determining the optimized parameters for improving toxin binding capacity of aflatoxin B1.

*Araştırma Makalesi*

# Aflatoksin B1 Bağlama Kapasitesinin Karışık Toksin Bağlayıcı ile Merkezi Kompozit Tasarım Kullanılarak Değerlendirilmesi

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ÖZ

Kanatlı beslenmesinde kullanılan karışık bir toksin bağlayıcı olan aflatoksin B1`in bağlama kapasitesi, merkezi kompozit tasarım tekniği kullanılarak belirlenmiştir. Karışım bağlayıcının bağlama kapasitesinin belirlenmesi için pH, sıcaklık ve inkübasyon süresi gibi deneysel koşulların optimizasyonu için merkezi kompozit tasarım kullanılmıştır. Bu üç bağımsız değişkenin aflatoksin B1'in bağlanma yüzdesi üzerindeki etkisi beş seviyede (-1.68, -1, 0, 1, 1.68) uygulanmıştır. Bu değişkenlerin 3 boyutlu grafikler dikkate alındığında aflatoksin B1 bağlanması üzerinde önemli etkiye sahip olduğu gözlenmiştir. Kuadratik model ve desirability fonksiyonu yardımıyla optimum deneysel koşullar pH 5.8, sıcaklık 42°C ve inkübasyon süresi 94.11 dakika olarak tespit edilmiştir. Her bir bağımsız değişken, aflatoksin B1'in bağlanma yüzdesi üzerinde anlamlı bir etkiye sahipti. Optimum deney koşullarında karışık toksin bağlayıcının aflatoksin B1'i %97 gibi yüksek bir oranında bağladığı belirlenmiştir. Sonuçlar, karışım bağlayıcının aflatoksin B1'in bağlanması için çok uygun olduğunu ve merkezi kompozit tasarımın toksin bağlayıcıların toksin bağlama kapasitesinin belirlenmesinde etkin bir şekilde kullanılabileceğini göstermiştir.

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Bu çalışmanın hazırlanma sürecinde bilimsel ve etik ilkelere uyulduğu ve yararlanılan tüm çalışmaların kaynakçada belirtildiği beyan olunur (M. Topkafa).

It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (M. Topkafa).

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**Çıkar Çatışması** **Conflict of Interest**

Yazarlar, bu makalede bildirilen çalışmayı etkiliyor gibi görünebilecek bilinen hiçbir rakip mali çıkarları veya kişisel ilişkileri olmadığını beyan ederler.

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## 1. Introduction

Mycotoxins are secondary metabolites produced by the growth of various fungal species under suitable environmental conditions (humidity, temperature, etc.) during processes such as harvesting and storage conditions, climate change, inappropriate drying and packaging (Milani, 2013; Mannaa and Kim, 2017; Kovač et al., 2018). Aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A, fumonisin B1 and trichothecenes (T-2 and HT-2) are the most important ones out of 400 identified mycotoxins in terms of their prevalence, economic aspects and negative effects on public health (Pitt, 2000; Eskola et al., 2020). While these mycotoxins can cause digestive disorders, oral lesions, immunological effects, hematological disorders and cancer, they exhibit many toxic effects, including teratogenicity, mutagenicity, nephrotoxicity, hepatogenicity, and genotoxicity (Frizzell et al., 2011; Bui-Klimke and Wu, 2015; Rushing and Selim, 2019). In mycotoxicosis, the severity of symptoms varies according to animal species, sex, age, exposure time, mycotoxin type, current level, and synergistic effect of these parameters by being present in more than one number (Schiefer, 1990).

Contamination of food and feed with mycotoxins is an important problem all over the world. They constitute a major risk factor for human and animal health. For this reason, various methods such as physical, chemical and biological are used in the detoxification of mycotoxins in order to minimize the negative effect (Gomaa et al., 1997; Raters and Matissek, 2008; Calado et al., 2014; Trombete et al., 2017; İPÇAK et al., 2019; Lyagin and Efremenko, 2019; Taheur et al., 2019). Binders, which are feed additives, are widely preferred by breeders and feed industry (De Mil et al., 2015). Binders are used to prevent clumping in feeds, but they also reduce toxin absorption from the digestive system by adsorbing mycotoxins. The toxin complex formed by toxin binders is then excreted in the feces. Toxin binders are basically classified as organic (yeast cell wall and glucomannan) and inorganic binders (clay minerals such as aluminosilicate, bentonite and zeolite) (Vila-Donat et al., 2018).

Feed additives can be used in animal nutrition to improve feed quality, performance and health of animals. These substances, which are scientifically proven to have no harmful effects on human and animal health and the environment, can be placed on the feed market with the permissions granted. The properties of the additives such as purity, physical properties (dusting potential, particle size, distribution, etc.), efficiency and reliability must be in the desired conditions (HUB; Commission, 1998; Additives et al., 2018). In the present work, central composite design as an experimental design was used to evaluation of % binding of aflatoxin B1 capacity for a mix toxin binder used in poultry nutrition.

## 2. Materials and Methods

### *2.1. Chemicals and standards*

The all chemicals in the analyzes were chromatographic purity and were purchased from Merck and Sigma-Aldrich. The toxin binder which has 20% yeast and 80% active clinoptilolite was provide from local market in Konya, Turkey. The aflatoxin B1 stock solution, which was purchased from Sigma-Aldrich, were prepared in amber bottle to 10 ppm and stored at +4°C until test was performed. Solutions that have different pH's were prepared with HCl and NaOH.

### *2.2. Experimental design and desirability function (DF)*

The central composite design parameters consisting of pH, time and temperature independent variables and experimental runs have 5 levels (-1.68, −1, 0, +1, +1.68) were given in Table 1. For response surface methodology (RSM), aflatoxin B1 was used as response value (RSD < 0.5%). The version 12 of the Design Expert software (Stat-Ease corporation, USA) was used for chemometric design. All samples were analyzed in triplicate (n= 3).

**Table 1.** CCD for the three independent variables.

| Run | pH | Temperature (°C) | Incubation Time (min) | % Binding |
|---|---|---|---|---|
| *1* | 6,69 | 41 | 95 | 87.14 |
| *2* | 4,5 | 41 | 69,7731 | 81.43 |
| *3* | 4,5 | 41 | 95 | 96.07 |
| *4* | 5,8 | 42 | 80 | 94.64 |
| *5* | 4,5 | 41 | 95 | 93.93 |
| *6* | 4,5 | 41 | 95 | 97.14 |
| *7* | 5,8 | 42 | 110 | 94.29 |
| *8* | 4,5 | 41 | 95 | 94.29 |
| *9* | 3,2 | 42 | 110 | 73.21 |
| *10* | 5,8 | 40 | 80 | 79.64 |
| *11* | 3,2 | 40 | 80 | 76.79 |
| *12* | 4,5 | 39,32 | 95 | 96.07 |
| *13* | 4,5 | 42,68 | 95 | 86.07 |
| *14* | 3,2 | 40 | 110 | 79.29 |
| *15* | 3,2 | 42 | 80 | 80.36 |
| *16* | 5,8 | 40 | 110 | 77.14 |
| *17* | 4,5 | 41 | 95 | 94.64 |
| *18* | 2,31 | 41 | 95 | 93.21 |
| *19* | 4,5 | 41 | 120,23 | 86.79 |
| *20* | 4,5 | 41 | 95 | 93.57 |

### *2.3. Sample collection and preparation*

10 mg of toxin binder was weighed into 15 mL falcon tube. 50 µL stock afl B1 standard solution (final concentration of 100 ppb) and 5 mL of the pH-adjusted solution were added and vortexed. The samples were shaken in the incubator with a stirrer at the specified temperature and time. Later samples were centrifuged at 1000 rpm/min for 3 minutes. After waiting for 10 minutes, 800µL of supernatant and 800µL of methanol were added to 2mL vials, vortexed and analyzed in HPLC.

### *2.4. HPLC and its parameters*

An Agilent 1260 series HPLC system consisted of florescence detector set at wavelengths 362 nm and 440 nm for excitation and emission, respectively, and on ACE C18 column (250× 4.6 mm, 5 µm,) was used for aflatoxin analysis. Photochemical derivatization (LC Tech -UVE) was used in

analysis. An isocratic separation of water, methanol and acetonitrile (57.2 /28.6/14.2 v/v/v/) was carried out at a flow rate of 1.0 ml $min^{-1}$ as mobile phase

## 3. Results and Discussion

Working samples containing afl B1 were prepared and incubated according to central composite design. After incubation, afl B1 analysis were carried out by using HPLC. The remaining afl B1 amount were utilized as response values and 3D plots were drawn. The optimum conditions for maximum afl B1 adsorption were detected by second-order quadratic model and desirability function.

The prepared and incubated samples were subjected to HPLC analysis. The % bindings of afl B1 were calculated by using the remaining Afl B1 amounts and were taken as response values. In order to determine the most accurate design after incubations under different conditions, the coded equation was calculated using the Design Expert software program. The design program determined that the quadratic model was the most suitable among 2FI, linear, quadratic and cubic models. While determining the suitable model, high R-square ($R^2$), appropriate coefficient of variation (CV) and low standard deviation (S.D) values were taken into account and the following equation was obtained:

$$AflB1=+95.1382+1.89293A+0.938874B+0.11089C+4.3325AB+0.225AC-0.9375BC-2.97997A^2-2.66353B^2-5.12427C^2$$

where A, B and C are pH, temperature and incubation time, respectively (Topkafa and Ayyildiz, 2017). Statistical results obtained from an analysis of variance (ANOVA) are shown in Table 2. The adjusted $R^2$ and *P* for % bindings of afl B1 was calculated calculated as 0.6230, 0.1787, respectively. The S.D value of 6.70 proved the performance of the model. The CV of 7.63% indicated that the model was reasonably reproducible as the CV was not greater than 10%. [23]. In terms of % bindings of afl B1 the model, F-value of 1.84 implies the model is not significant. The Lack of Fit F-value of 46.06 implied the Lack of Fit is not significant relative to the pure error. There is only a 0.03% chance that a Lack of Fit F-value this large could occur due to noise.

**Table 2.** ANOVA for the model predicted.

| Source | SS | DF | MS | F-value | p-Value | Comment | $R^2$ | Std. Dev. | C.V. % |
|---|---|---|---|---|---|---|---|---|---|
| Model | 740.78 | 9 | 82.31 | 1.84 | 0.1787 | not significant | | | |
| Error (Residual) | 448.29 | 10 | 44.83 | | | | | | |
| Lack of Fit | 438.77 | 5 | 87.75 | 46.06 | 0.0003 | significant | 0.6230 | 6.70 | 7.63 |
| Pure Error | 9.53 | 5 | 1.91 | | | | | | |
| Total | 1186.08 | 19 | | | | | | | |

By using regression models, the 3D surface plots were plotted for % bindings of afl B1. The 3D plots indicate the effects of pH, incubation time and temperature on % binding of afl B1. In 3D plots (Fig 1), one factor is kept constant at the center (0) while the other two factors are changed. The effect of pH (A) and temperature (B) on % bindings of afl B1 (Y), while incubation time (C) at the center point, are shown in Fig. 1a. Fig. 1b shows effect of pH (A) and incubation time (C) on % bindings of afl B1 (Y), while temperature (B) at the center point. Fig. 1c shows effect of temperature (B) and incubation time (C) on % bindings of afl B1 (Y), while pH (A) at the center point. Corresponding to these plots, pH, time and temperature had a relevant effect on % bindings of afl B1. In studies where the incubation time was kept at the central point, the % binding of afl B1 on toxin binder increased at high temperature and pH values.

The present study aimed to determine quickly the optimum % bindings of afl B1 with fewer experiments. For this reason, desirability function (D) was used for determining the optimum experimental conditions (Fig. 2). The desirability function (D) can be used to deal with the optimization of multiple response problems in which too many variables are affected. The desirable ranges according to the goal can be changed from zero to one or target value. The target value was set at maximize for % bindings of afl B1. Desirability values (optimum experimental conditions) of maximize for % bindings of afl B1 were found to be pH 5.8, temperature 42°C and incubation time 94.11 min by using desirability function. In the study which was carried out under optimum experimental conditions, % bindings of afl B1 was found to be 97%.

Many articles have been published about the binding of afl B1 in chicken feeds on inorganic toxin binders such as active clinoptilolite and bentonite and organic toxin binders such as glucomannan and yeast (Diaz et al., 2003; Faucet-Marquis et al., 2014; Bočarov-Stančić et al., 2018; Yalcin et al., 2018; Ahn et al., 2022). These studies are usually focused on single toxin binding studies. The mix binder including clinoptilolite and yeast was used in our study. While the binding capacity of zeolite containing clinoptilolite was 21.9 % in the study performed by Vekiru et al. (2015), the binding capacity of zeolite was found to be 95.5% in a study conducted by Bočarov-Stančić et al. (2018). Faucet-Marquis et al. (2014) and Ahn et al. (2022) found that max % bindings of afl B1 were 70% and 92.3%, respectively, in the studies in which yeast was used as binder. However, in our study where a mixture binder containing clinoptilolite and yeast was used, the maximum max % bindings of afl B1 was found to be 97%. The reason for the higher % bindings of afl B1 is thought to be due to the synergistic effect of both organic yeast and inorganic clinoptilolite binders.

## 4. Conclusions

In this study, % binding capacity of afla B1 of mixed toxin binder used as toxin binder in poultry nutritions was evaluated using central composite design technique. pH, temperature and incubation time were used as variables in the binding capacity experiments and it was observed that these variables they had a significant effect on afl B1 binding when taking into consideration to 3D plots. With the help of the proposed quadratic model, ANOVA, desirability function and 3D graphics, it was found that the optimum experimental conditions for the highest % binding capacity of afl B1 were pH 5.8, temperature 42°C and incubation time 94.11 min. It was observed that the mixed toxin binder binds 97% afl B1 when optimum experimental conditions were used. It was found that mixed toxin binder had binding of afl B1 at a high rate. As a conclusion, it has been seen that the CCD can be used effectively in determining the toxin binding capacity of toxin binders.

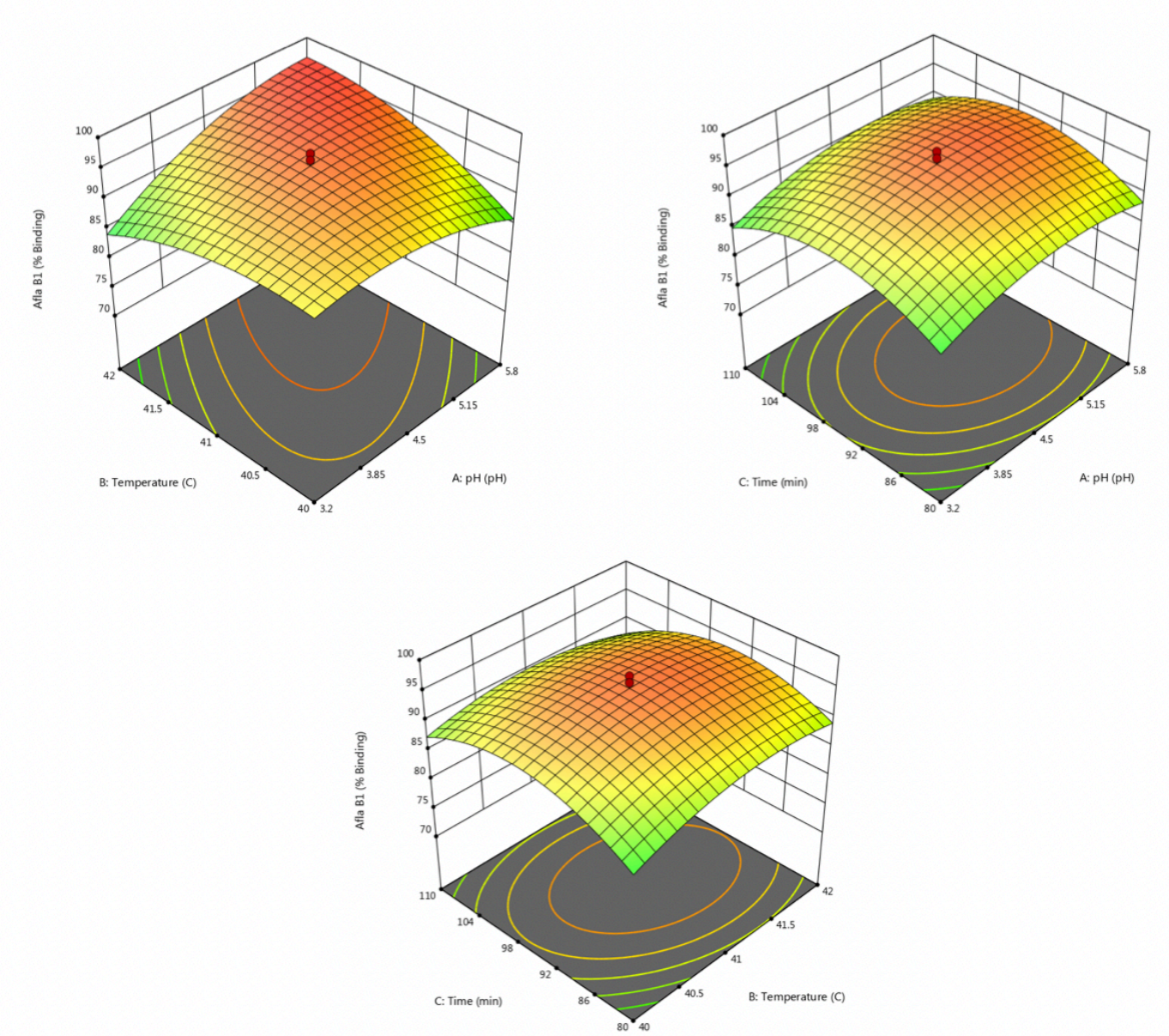


**Fig. 1.** The three-dimension response surface plots (3D) and normal plot of the residuals of % Binding (Y), A: pH; B: Temperature ( °C); C: Incubation Time (min).

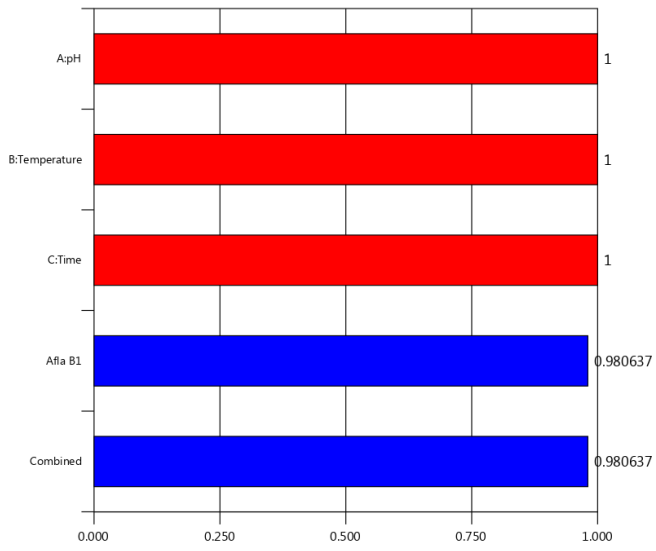


**Fig. 2.** Desirability bar graph.

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## CRediT author statement

NFY, CC and MT conceived and HO and STHS supervised this study. NFY, CC and MT completed the main experimental content. NFY and CC and MT collected and analyzed data. MT and MSA wrote the first draft of the article. All authors contributed to the critical revision of the article and have read and approved the final version.

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*Research Article*

# Electrochemical Properties and Determination of Serotonin with Graphene/Coal Tar Pitch/Pencil Graphite Sensor Electrode using Square Wave Adsorptive Stripping Voltammetry Technique

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ABSTRACT

In the present work, electrochemical and spectroelectrochemical behaviors of Serotonin (5-HT) were studied by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. Cyclic voltammetric experiments of 5-HT on graphene/coal tar pitch/pencil graphite electrode (GR/CTP/PGE) were carried out between 0.0 V and 1.8 V potential range at a scan rate of 100 mV $s^{-1}$ with 20 cycles in non-aqueous media. Surface characterizations were performed using CV, EIS and scanning electron microscope (SEM). Effect of different pH values was investigated by square wave voltammetry (SWV) for determination of 5-HT. Optimization of accumulation time was determined using square wave adsorptive stripping voltammetry (SWAdSV) within potential range of -0.2 to +0.6 V. 5-HT standard solutions changing from 75 µM to 1.0 µM were prepared and the corresponding peak currents were measured. From the obtained data calibration equation was derived $Ip = 0.0329C_{5\text{-HT}} + 0.1511$ with correlation coefficient ($R^2$) 0.9958. LOD was $3.51\text{x}10^{-7}$ M and LOQ was $1.05\text{x}10^{-6}$ M.

*Araştırma Makalesi*

# Kare Dalga Adsorptif Sıyırma Voltametrisi Tekniği Kullanılarak Grafen/Katran/Kalem Grafit Sensör Elektrot ile Serotonin'in Tayini ve Elektrokimyasal Özellikleri

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ÖZ

Bu çalışmada Serotonin'in (5-HT) elektrokimyasal ve spektroelektrokimyasal davranışları dönüşümlü voltametri (CV) ve elektrokimyasal impedans spektroskopisi (EIS) teknikleriyle incelenmiştir. Grafen/katran/kalem grafit elektrot (GR/CTP/PGE) üzerinde 5-HT'nin CV deneyleri, 0,0 V ile 1,8 V potansiyel aralığında sulu olmayan ortamda $Ag/Ag^{+}$/(10 mM $AgNO_3$) referans elektrotu kullanılarak 20 döngü ile 100 mV $s^{-1}$ tarama hızında gerçekleştirildi. Yüzey karakterizasyonları CV, EIS ve taramalı elektron mikroskobu (SEM) kullanılarak yapıldı. 5-HT'nin tayininde farklı pH değerlerinin etkisi kare dalga voltametri (SWV) tekniği ile araştırıldı. Biriktirme süresinin optimizasyonu, -0,2 ile +0,6 V potansiyel aralığında kare dalga adsorptif sıyırma voltametrisi (SWAdSV) kullanılarak belirlendi. 75 µM'den 1,0 µM'ye kadar değişen 5-HT standart çözeltileri hazırlandı ve karşılık gelen pik akımları ölçüldü. Elde edilen verilerden kalibrasyon denklemi $Ip = 0{,}0329C_{5\text{-HT}} + 0{,}1511$ ve korelasyon katsayısı ($R^2$) 0,9958 olarak hesaplandı. LOD $3{,}51\text{x}10^{-7}$ M ve LOQ $1{,}05\text{x}10^{-6}$ M olarak belirlendi.

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Yazarlar, bu makalede bildirilen çalışmayı etkiliyor gibi görünebilecek bilinen hiçbir rakip mali çıkarları veya kişisel ilişkileri olmadığını beyan ederler. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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## 1. Introduction

Serotonin, or 5-hydroxytryptamine (5-HT), serves as a crucial signaling molecule within both animals and plants (Szeitz & Bandiera, 2018). Investigating neurotransmitters like 5-HT remain a highly dynamic area of research, particularly in the context of addressing chronic conditions such as Alzheimer's and Parkinson's diseases. These neurotransmitters hold pivotal roles in regulating the central nervous system, with deviations in their levels being implicated in various neurological disorders. The normal 5-HT level in humans is reported as 0.25-0.74 mM by the US Department of Health, Human Services, and NIH. Increase or decrease in the normal concentration of 5-HT can cause depression, migraines, anxiety, eating disorders, sudden infant death syndrome (SIDS) and blood clots, etc. (Koluaçık et al., 2018).

Electrode modification can be widely performed in aqueous and non-aqueous media (Islamoglu et al., 2023; Demir Mülazımoğlu & Mülazımoğlu, 2013; Demir Mülazımoğlu et al., 2012). Conducting polymers are widely employed as modifiers in fabrication of modified electrodes. In this context, modification of glassy carbon (GC) electrode with gold nanoclusters/overoxidized polypyrrole films has been carried out to prepare poly(pyrrole-3-carboxylic acid) films for electrochemical detection of 5-HT (Li & Lin, 2007). Surface deactivation due to polymerization or adsorption of oxidized molecules on electrode surface is crucial disadvantages of modified electrodes. Various electrodes such as GC electrode (Albayrak et al., 2017), carbon paste electrode (Mülazımoğlu & Yılmaz, 2010), PGE (Korkmaz et al., 2023) were used to determination of different species. For this reason, pencil can be regarded as a significant contender for disposable electrodes due to its cheapness and easy to use (Durmuş & Mülazımoğlu, 2023; Özcan & Ilkbas, 2015).

Development of a rapid, sensitive, and selective 5-HT determination method is very important and vital in terms of health and clinical treatment. Electrochemical methods stand out as the most promising techniques, thanks to their high selectivity and low detection limits in contrast to traditional chromatography and spectroscopy methods. Nevertheless, the efficacy of electrochemical methods hinges largely on the choice of working electrode employed in measurements. Conventional carbon and metal electrodes, typically, exhibit subpar performance when it comes to detecting 5-HT. For this reason, there is a trend of substituting pencils with alternative materials (Özcan & Ilkbas, 2015). For determination of 5-HT, different analytical methods have been used such as; differential pulse voltammetry (Kim et al., 2012), SWV (Motsaathebe & Fayemi, 2021), fluorometry (Mumtaz et al., 1982), high-performance liquid chromatography (Kema et al., 2000), chemi-luminescence (Barnett et al., 1998) and mass spectrometry (Yilmaz et al., 2019).

In this study, electrochemical and spectro electrochemical behaviors of 5-HT were investigated on modified GR/CTP/PGE surface using CV and EIS techniques. 5-HT modified GR/CTP/PGE was characterized by CV, EIS and SEM. The scanning speed of GR/CTP/PGE was investigated using LSV technique to achieve diffusion-controlled transfer to the electrode surface. The detection ability of 5-HT was investigated using SWAdSV technique.

## 2. Material and Method

### *2.1. Chemicals and reagents*

All chemicals and reagents were obtained from sources of analytical purity, including Sigma-Aldrich, Alfa Aesar, and Merck. Phosphate buffer solution (PBS) of different pH levels was formulated by combining standard solutions of $KH_2PO_4$ and $K_2HPO_4$, with pH adjustments made using 0.1 M NaOH solution. Ultra-pure distilled water with resistance of 18.2 MΩ cm was utilized in the preparation of all aqueous solutions. (MP MINIPURE Purification System, DEST UP).

### *2.2. Electrodes and equipments*

GR/CTP/PGE was prepared in accordance with the literature and was used as working electrodes (Üstündag & Erkal, 2017). While BAS MF-2063 model Ag/AgCl 3M KCl(sat) was used as aqueous media reference electrode, BAS MF- 2042 model Ag/$Ag^+$/(10 mM $AgNO_3$) was used as non-aqueous media reference electrode. Platinum wire electrode obtained from BAS model MW-1032 served as auxiliary electrode. Electrochemical experiments were performed using the traditional three-electrode system. CV, SWV, LSV, EIS and SWAdSV studies were performed on a reference GAMRY Reference 600+ potentiostat/galvanostat/ZRA. SEM studies were conducted using a Hitachi-SU 1510. The pH value in aqueous solutions was determined using a pH meter equipped with a glass composite pH electrode (VWR pH enomenal, UK). Room temperature (25 ± 1 °C) was chosen as the most appropriate parameter for all experiments.

## 3. Results and Discussion

### *3.1. Modification of GR/CTP/PGE with 5-HT*

The modification process of GR/CTP/PGE was carried out using the 5-HT via alcohol oxidation method with the CV technique. CV experiments were executed 1 mM 5-HT solution prepared in a 100 mM $NBu_4BF_4$ solution dissolved in $CH_3CN$, in potential range of 0.0 V to 1.8 V at a scan rate of 100 mV $s^{-1}$ with 20 cycles in non-aqueous media *vs.* Ag/$Ag^+$/(10 mM $AgNO_3$ in $CH_3CN$).

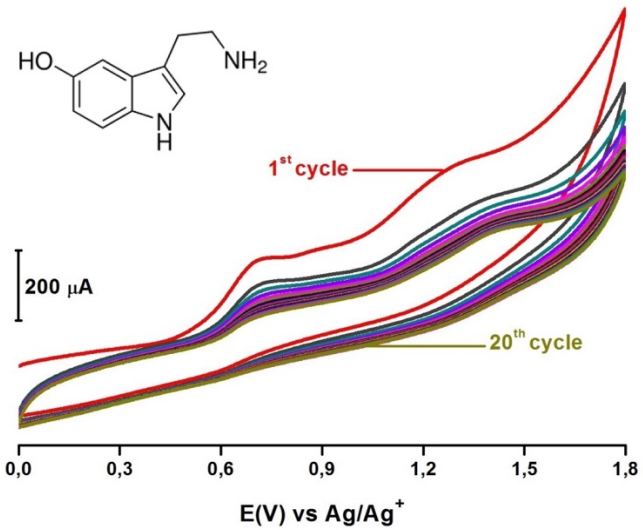


**Figure 1.** CV voltammogram of the modification of 5-HT on GR/CTP/PGE surface in the range from 0.0 to 1.8 for 20 cycles.

When the modification voltammogram given in Figure 1 was examined, peaks corresponding to oxidation of $-NH_2$ moiety at approximately 0.7 V and, subsequently, covalent bonding of C-O at around 1.2 V were observed. In the modification voltammogram, it was clearly seen that the peaks decrease starting from second cycle. The modification process was carried out in 20 cycles to prevent small gaps called pin holes from remaining.

### *3.2. Characterization of 5-HT/GR/CTP/PGE surface by CV, EIS and SEM*

In non-aqueous media, a redox probe solution was prepared using a 1 mM ferrocene solution in a 100 mM $NBu_4BF_4$ supporting electrolyte solution prepared in $CH_3CN$. In the prepared solution, CV was performed on GR/CTP/PGE and 5-HT/GR/CTP/PGE surface in potential range of -0.1 V to 0.6 V at a scan rate of 100 mV $s^{-1}$ with one cycle against an $Ag/Ag^+$/(10 mM $AgNO_3$) reference electrode. Voltammograms were obtained for both bare GR/CTP/PGE and 5-HT-modified GR/CTP/PGE surface under the same conditions and these voltammograms were presented as overlayed in Figure 2. The higher peak currents observed for oxidation and reduction peaks on 5-HT/GR/CTP/PGE surface compared to GR/CTP/PGE surface indicate the increased activity of the modified electrode surface.

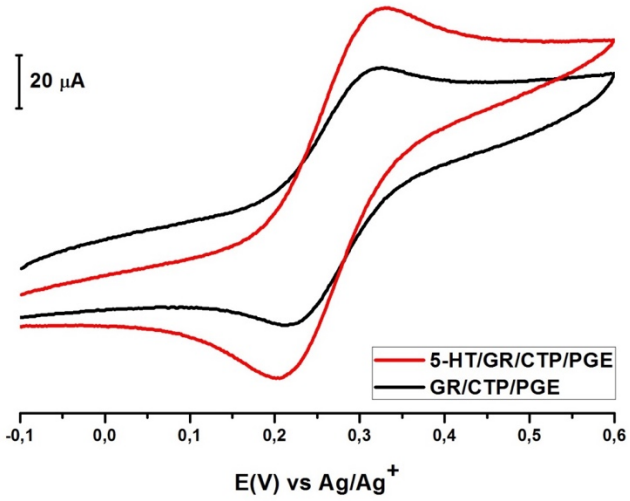


**Figure 2.** Cyclic voltammograms of 1.0 mM ferrocene redox probe, potential range from -0.1 to 0.6 V on bare GR/CTP/PGE and 5-HT/GR/CTP/PGE's surfaces.

To support the characterization study performed in non-aqueous media, 1 mM solution of HCF(III) redox probe was prepared by dissolving it in pH 2.00 BR buffer in the aqueous media. CV was conducted within potential range of 0.5 V to -0.2 V at a scan rate of 100 mV $s^{-1}$, comprising a single cycle against Ag/AgCl 3M KCl(sat) reference electrode.

Voltammograms were obtained for both bare GR/CTP/PGE and 5-HT/GR/CTP/PGE surface. These voltammograms were presented as overlayed in Figure 3. Similar to the characterization conducted in non-aqueous media, higher peak currents on the modified surface were observed in aqueous media using the CV technique.

Following the electrochemical characterization processes performed using CV technique, impedance measurements were carried out using another electrochemical technique, EIS with a 100 mM KCl solution including a $Fe(CN)_6^{3-/4-}$ redox probe.

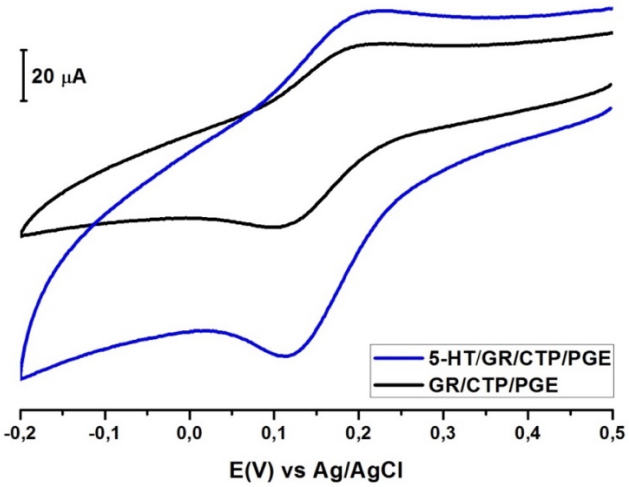


**Figure 3.** Cyclic voltammograms of 1 mM HCF(III) redox probe, potential range from 0.5 V to -0.2 V on GR/CTP/PGE and 5-HT/GR/CTP/PGE's surfaces.

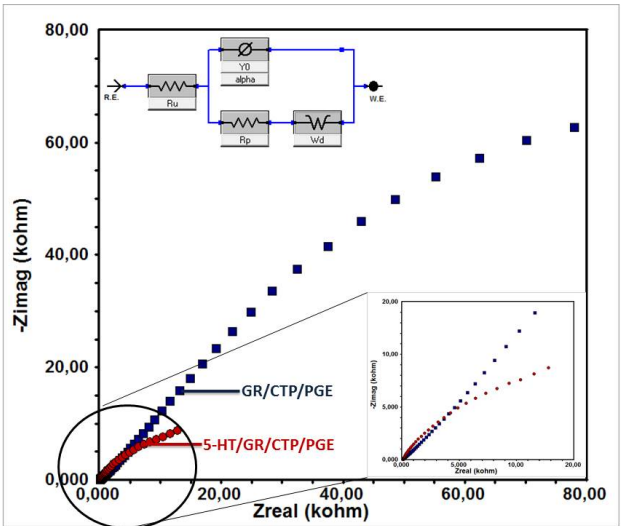


**Figure 4.** Nyquist plots for electrochemical impedance spectra of 1 mM $Fe(CN)_6^{3-/4-}$ redox couple solution in 100 mM KCl on GR/CTP/PGE and 5-HT/GR/CTP/PGE's surfaces at frequency range of 100 000–0.01 Hz at 10 mV wave amplitude.

The obtained Nyquist plots were compared between the modified electrode and GR/CTP/PGE. The graph representing this comparison is provided in Figure 4. When 5-HT/GR/CTP/PGE was compared with GR/CTP/PGE surface, it was shown 5-HT/GR/CTP/PGE surface was more active.

After the electrochemical characterization studies, microscopic images of the surfaces were obtained using SEM technique. The acquired SEM images were presented in Figure 5A, representing the image of GR/CTP/PGE surface, and Figure 5B, representing the image of 5-HT/GR/CTP/PGE surface. Morphologically, the difference between two images serves as sufficient evidence for modification of electrode surface. These images were obtained by scanning approximately 1 µm areas magnified 5000 times.

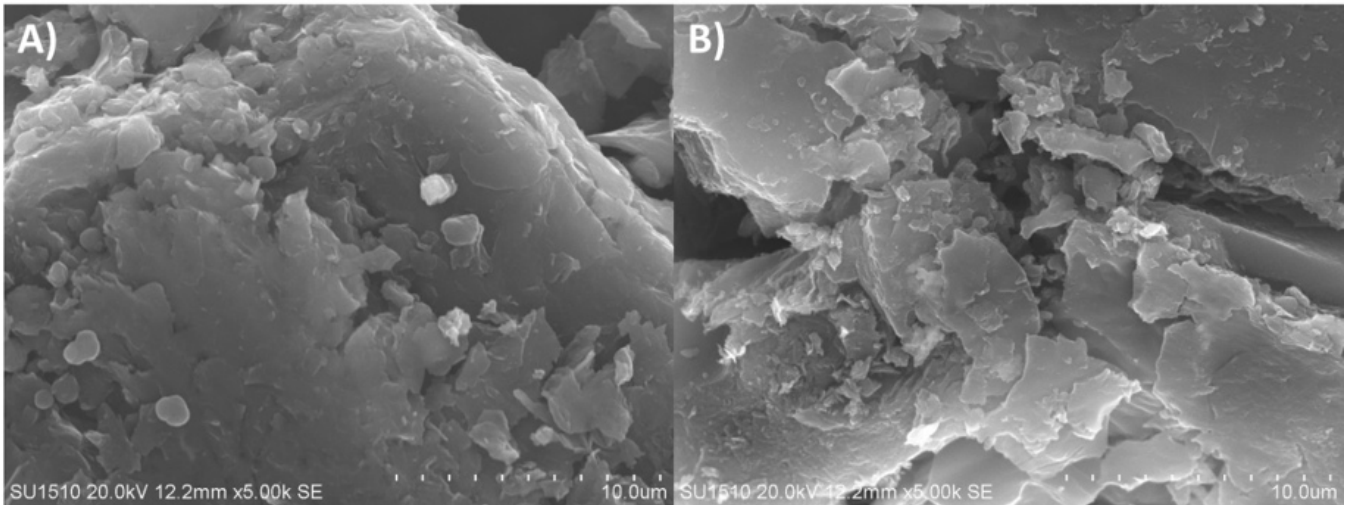


**Figure 5.** SEM images of A) GR/CTP/PGE and B) 5-HT/GR/CTP/PGE surfaces.

### *3.3. Effect of scan rate*

The surfaces, resulting from characterization processes, were assessed, leading to the presumption the surface obtained following modification on GR/CTP/PGE was deemed suitable for intended application. To determine whether molecule reaches electrode surface electrochemically in a diffusion-controlled manner, scan rate studies were conducted. In these studies, 1 mM 5-HT solution prepared in a 100 mM $NBu_4BF_4$ support electrolyte in $CH_3CN$ was used. The study was conducted using LSV and overlayed voltammograms were presented in Figure 6A. The voltammograms were obtained at scan rates of 10, 25, 50, 100, 150, 200, 250 and 300 mV $s^{-1}$. In line with Equation 3.1 of the Randles-Sevcik equation, the graph's linearity, depicting square root of scan rates against peak current, suggests that the molecule binds to electrode surface under diffusion-controlled conditions.

$$i_p = 2.69x10^5 n^{3/2} A D^{1/2} C v^{1/2} \quad \text{(Equation 3.1)}$$

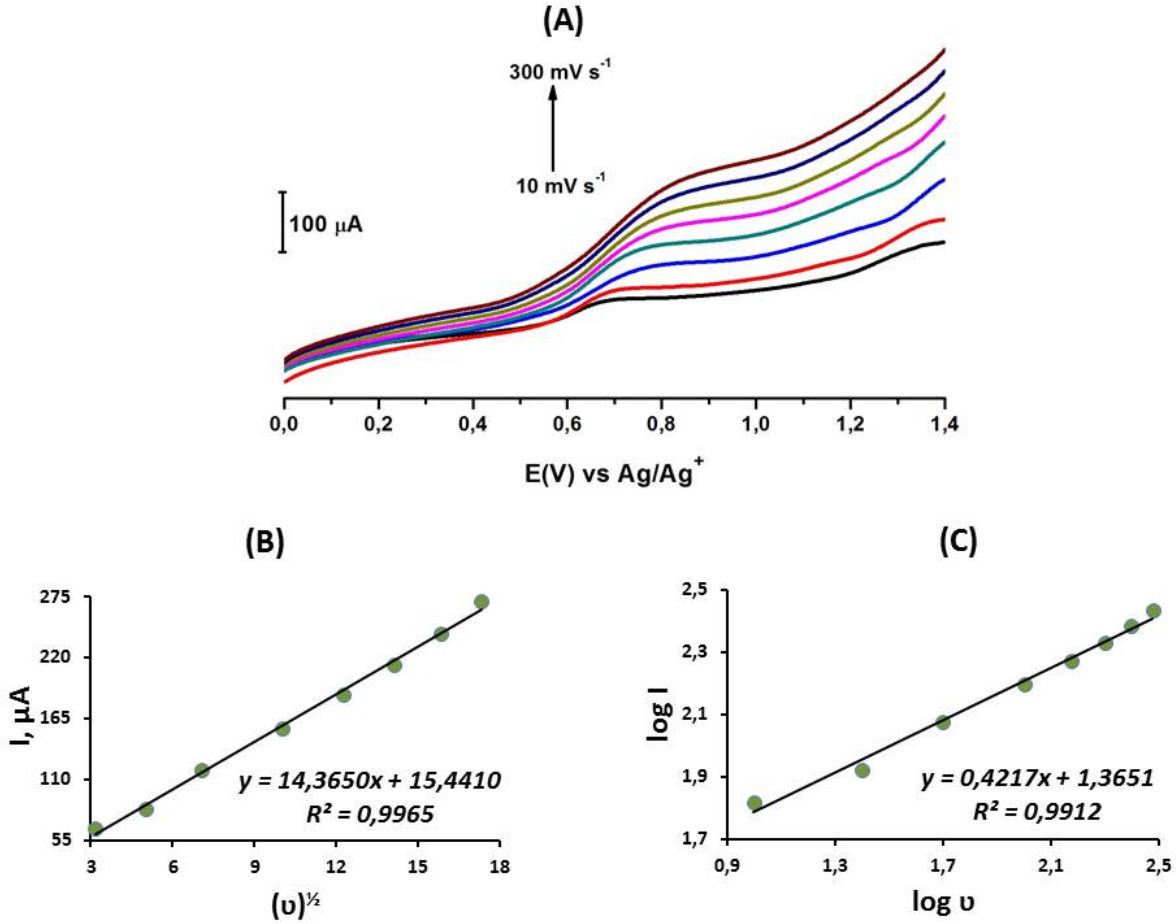


**Figure 6.** A) LSV oxidation voltammograms of 1 mM 5-HT on GR/CTP/PGE surface at various scan rates of 10, 25, 50, 100, 150, 200, 250 and 300 mV $s^{-1}$ at the potential range from 0 to +1.4 V. B) Shows plot of peak current vs. square root of scan rate. C) A plot of log scan rate vs. log peak current.

The correlation coefficient, $R^2$=0.9965, for the obtained line in Figure 6B suggested a strong correlation for the linear fit. Additionally, slope of the plot of log Ip against log ν in Figure 6C was approximately 0.5, indicating molecule was transported to electrode surface in a diffusion-controlled.

### *3.4 Effect of pH*

The impact of pH of the solution is important in determining 5-HT. As the physiological pH of the human body is pH 7.4, in this study, 0.1 M PBS was used in various pH ranges (pH 6.8, 7.2, 7.6 and 8.0) with SWV. SW voltammograms were given (Figure 7A) and optimum pH was determined. The response of GR/CTP/PGE to 5-HT (1 mM) at different pH values were shown in Figure 7B.

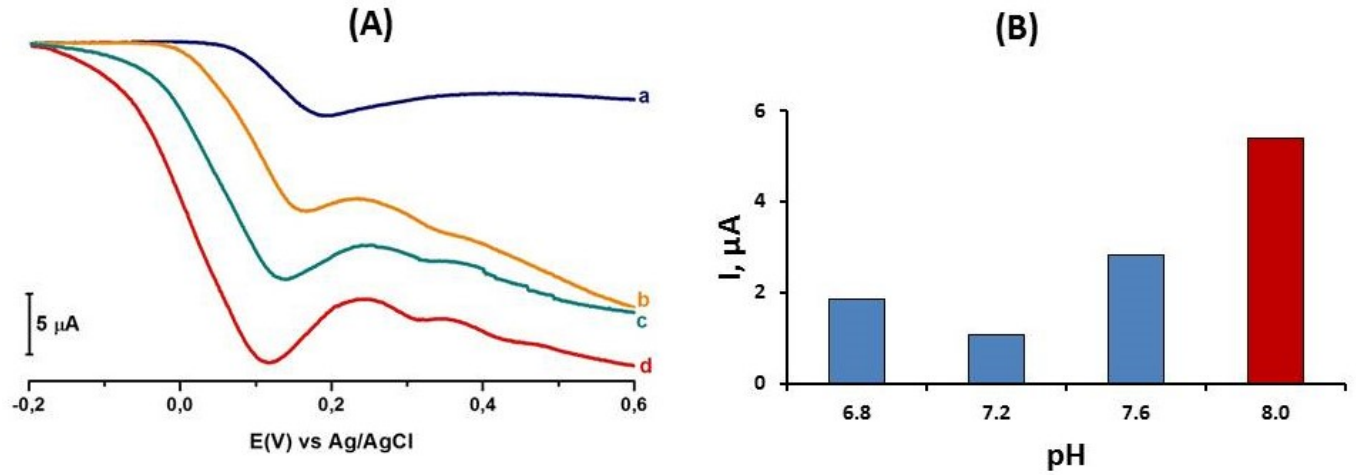


**Figure 7.** A) SW voltammograms obtained on GR/CTP/PGE surface of 1 mM 5-HT solution prepared at various pH of 0.1 M PBS (a) 6.8, b) 7.2, c) 7.6, d) 8.0). B) Peak current values for 5-HT in PBS with various pH on GR/CTP/PGE.

### *3.5. Optimization of accumulation time*

Experimental conditions involved conducting current measurements using SWAdSV within potential range of -0.2 to +0.6 V. In Figure 8A, the voltammograms obtained from measurements taken within the 0 to 90 seconds range under optimal conditions were overlayed. Additionally, a graph (Figure 8B) depicting relationship between peak current and accumulation time was plotted. From this graph, it was determined that an accumulation time of 30 seconds appropriate for this study.

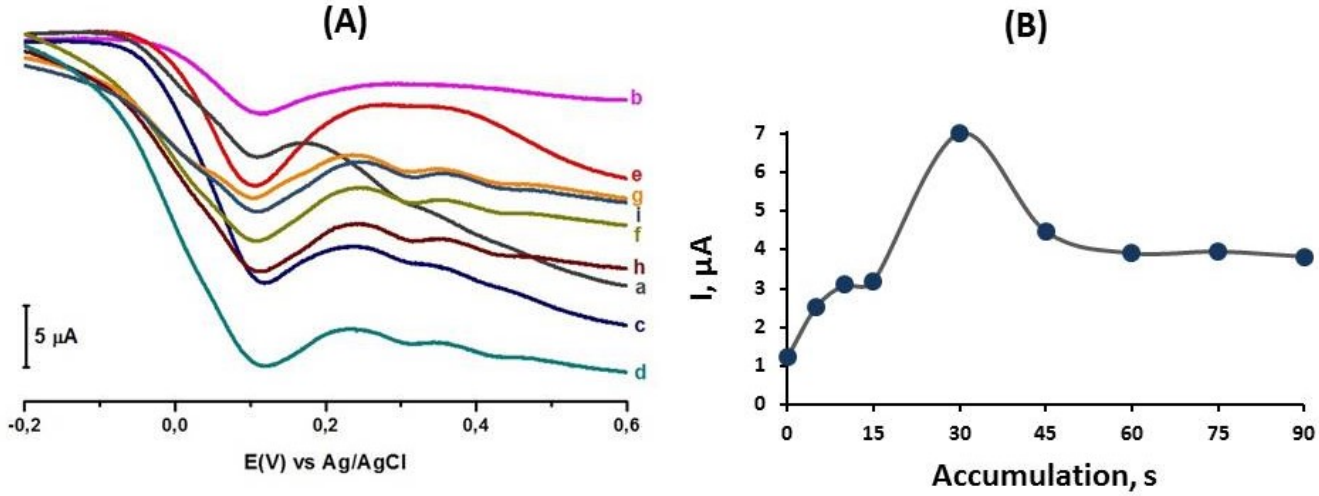


**Figure 8.** A) SWAdSV's obtained on GR/CTP/PGE surface of 1 mM 5-HT solution prepared in 0.1 M PBS pH 8.0 solution and potential range of -0.2/+0.6 V, for various accumulation times (0, 5, 15, 30, 35, 45, 60, 75, 90 s), B) Graph plotted with peak current values against accumulation time.

### *3.6. Analytical performance of 5-HT*

The analytical performance of GR/CTP/PGE surface was evaluated by constructing a calibration curve at different concentrations of 5-HT (75, 50, 25, 10, 5, 1 µM) in PBS at pH 8.0 solution using SWAdSV. Voltammograms were taken at different concentrations and these voltammograms were superimposed in Figure 9A. Figure 9B was shown appearance of linear regression between peak current variation and 5-HT concentration. From the obtained data calibration equation was calculated $Ip = 0.0329C_{5\text{-HT}} + 0.1511$ with linear correlation coefficient ($R^2$) 0.9958. LOD was $3{,}51 \times 10^{-7}$ M and LOQ was $1{,}05 \times 10^{-6}$ M.

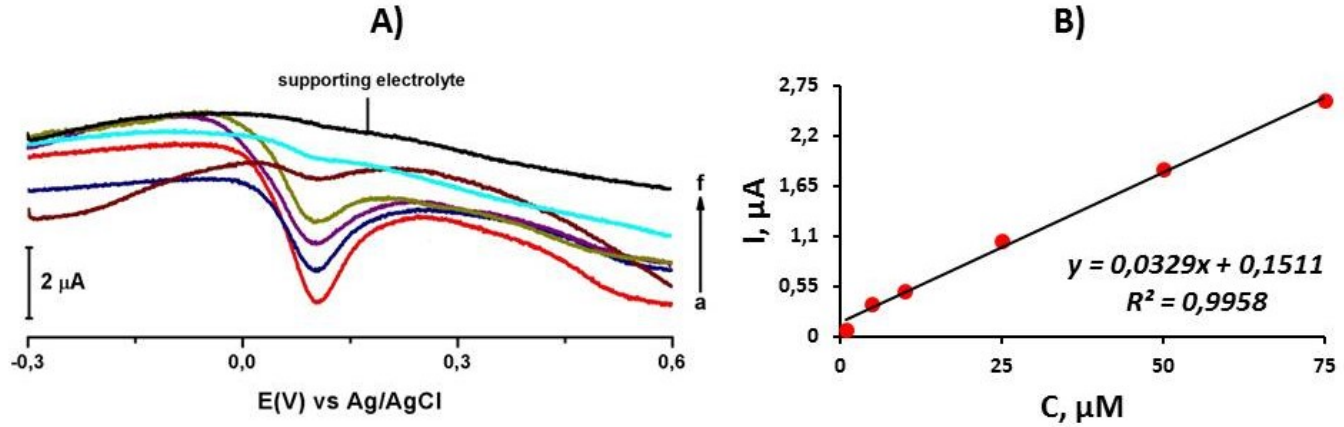


**Figure 9.** A) Overlaying SWAdSV's for solutions prepared various concentrations of 5-HT (a) 75, b) 50, c) 25, d) 10, e) 5, f) 1 µM) in the 0.1 M PBS at pH 8.0 on GR/CTP/PGE (Accumulation time 30 s and stirring rate 350 rpm) and B) Linear calibration curve of peak current versus 5-HT concentration in SWAdSV measurements.

## 4. Conclusions and Recommendations

In this study, GR/CTP/PGE synthesized in accordance with the literature, which has a low cost, renewable, broad, and electroactive surface, was applied as the working electrode for all analysis.

It can be clearly seen 5-HT binds on the surface after modification with the CV technique in non-aqueous media, through CV tests performed with ferrocene redox probe in non-aqueous media, with HCF(III) redox probe in aqueous media, and through impedance tests performed with EIS using $Fe(CN)_6^{3-/4-}$ redox probe in aqueous media.

In addition, surface characterization was obtained using SEM technique. With SEM technique, it can be seen bare GR/CTP/PGE is a bulk structure and 5-HT/GR/CTP/PGE is a layered structure. According to the results obtained from scanning rate studies, 5-HT is transported to electrode surface under diffusion control.

After determining the optimum parameters, concentration-peak current calibration graph drawn using 5-HT solutions prepared at different concentrations (75, 50, 25, 10, 5, 1 µM) in pH 8.0 PBS evidenced by a correlation coefficient of 0.9958.

Proposed method provides a simple, rapid and economical to determination of 5-HT using GR/CTP/PGE by SWAdSV technique. Overall, this research provides a comprehensive analysis of electrochemical behaviors of 5-HT, offering valuable insights into its detection and quantification, which could have significant implications in various fields such as neuroscience and pharmaceuticals. In future studies, trace amounts of 5-HT in natural or synthetic samples can be easily determined.

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## CRedit author statement

ŞK: Experimental work and data analysis, drawing of figures in the article, carried out certain experiments data plotting and analysis.
HS: Experimental work, data curation and writing the introduction and experimental part of the article.
ADM: Facilitation in the experimental work, technical discussion, Writing – review & editing.

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*Research Article*

# Investigation of Electrochemical Properties and Behaviors of NBITEP Molecule; Availability of Sensor Electrode in Determination of $Hg^{2+}$ with SWAdSV Technique

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ABSTRACT

This Master's Thesis focuses on the synthesis and structural elucidation of 4-(2-((6-nitro-1h-benzo[d]imidazol-2-yl)thio)ethyl)phenol (NBITEP) molecule, along with its electrochemical behaviors. Electrochemical studies were carried out employing a glassy carbon (GC) electrode as the working electrode. The cyclic voltammetry (CV) technique was utilized for modification and characterization processes. Furthermore, the potential of the modified surface as a sensor electrode for $Hg^{2+}$ ion detection was investigated using square wave adsorptive stripping voltammetry (SWAdSV). Detailed examinations were conducted in a phosphate buffer solution (PBS) medium specifically for $Hg^{2+}$ ion detection, demonstrating the suitability of the reduced NBITEP modified GC electrode surface as a sensor electrode.

*Araştırma Makalesi*

# NBITEP Molekülünün Elektrokimyasal Özelliklerinin ve Davranışlarının İncelenmesi; SWAdSV Tekniği ile $Hg^{2+}$'nın Tayininde Sensör Elektrotun Kullanılabilirliği

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ÖZ

Bu çalışmada, 4-(2-((6-nitro-1h-benzo[d]imidazol-2-il)tiyo)etil)fenol (NBITEP) molekülü sentezlenmiş, karakterize edilmiş ve çalışma bir Yüksek Lisans Tezi olarak sunulmuştur. Elektrokimyasal incelemelerde camsı karbon (GC) elektrot, çalışma elektrodu olarak kullanılmıştır. Modifikasyon ve karakterizasyon işlemlerinde dönüşümlü voltametri (CV) tekniğinin kullanıldığı çalışmada, modifiye edilmiş yüzeyin $Hg^{2+}$ iyonu için bir sensör elektrot olarak kullanılabilirliği, kare dalga adsorptif sıyırma voltametri (SWAdSV) tekniği ile araştırılmıştır. Fosfat tampon çözeltisi (PBS) ortamında gerçekleştirilen çalışmalar, $Hg^{2+}$ iyonu için indirgenmiş NBITEP modifiye GC elektrotun bir sensör elektrot olarak kullanılabileceğini ortaya koymuştur.

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Bu çalışmanın hazırlanma sürecinde bilimsel ve etik ilkelere uyulduğu ve yararlanılan tüm çalışmaların kaynakçada belirtildiği beyan olunur (İ.E. Mülazımoğlu). It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (İ.E. Mülazımoğlu).
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Yazarlar, bu makalede bildirilen çalışmayı etkiliyor gibi görünebilecek bilinen hiçbir rakip mali çıkarları veya kişisel ilişkileri olmadığını beyan ederler. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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## 1. Introduction

The investigation of molecules with desirable electrochemical properties is crucial for the development of advanced sensing platforms. In this context, the NBITEP molecule has garnered attention due to its intriguing structural features and potential applications in electrochemical sensing. This article aims to elucidate the electrochemical behavior of NBITEP and explore its utility as a sensor electrode for the detection of $Hg^{2+}$ ions, which pose significant environmental and health risks.

Electrochemical methods offer distinct advantages over traditional spectrophotometric techniques, including cost-effectiveness, in situ and continuous monitoring, portability, and straightforward instrumentation, often requiring minimal sample pretreatment.

Multiwall carbon nanotubes, categorized as innovative carbon materials, find widespread use across various domains, particularly in electrochemical sensing. This popularity is attributed to their high porosity, hollow structure, exceptional electrical conductivity, substantial specific surface area, and favorable biological activity (Hu et al., 2011; Islamoğlu et al., 2023; Korkmaz et al., 2023). Despite the many superior characteristics of multiwall carbon nanotubes, there is ongoing interest in enhancing their unique performance further. The goal is to fully exploit their extensive specific surface area and high loading capacity to support other nanoparticles. Combining multiwall carbon nanotubes with nanomaterials, such as metal nanomaterials, has been shown to provide a substantial specific surface area, excellent electrical conductivity, and biological compatibility (Leventis et al., 2005; Zheng et al., 2008), facilitating accelerated electron transfer between biological molecules and the electrode surface.

Recent findings underscore the suitability of carbon nanomaterials, including carbon nanotubes, graphene, carbon nanopowders, or nanofibers, in the preparation of electrochemical sensors due to their large active surface area and superior electrical conductivity (Li et al., 2013). Graphene-based materials, with their distinctive structure, have garnered significant attention in recent years. The conjugated, one-atom-thick, two-dimensional structure imparts excellent electron transport mobility, mechanical strength, and thermal stability to graphene (Pumera et al., 2010; Shao et al., 2010), enhancing its potential applications in electrochemical sensors (Mülazımoğlu & Mülazımoğlu, 2013; Mülazımoğlu & Mülazımoğlu, 2012; Mülazımoğlu et al., 2011).

## 2. Material and Method

Experimental procedures involved the synthesis and characterization of NBITEP, followed by the modification of electrode surfaces and electrochemical studies using cyclic voltammetry. The SWAdSV technique was employed for the sensitive determination of $Hg^{2+}$ ions, utilizing reduced NBITEP modified GC electrode surfaces. Comprehensive analyses were conducted in PBS media to evaluate the sensor performance and detection capabilities.

### *2.1. Instruments and chemicals*

All the chemicals were used from Fluka, and Sigma-Aldrich, all chemicals were used without any purification. Three electrodes electrochemical cell setup supplied with bare GC as working electrode, Pt wire (BAS Model MW-1032) as counter electrode and Ag/AgCl/ 3M KCl (BAS Model MF-2063) as reference electrode. Reference 600+ Potentiostat/Galvanostat/ZRA from Gamry (USA) was used in all measurements. Bare GC with a geometric area of 0.071 $cm^2$ or NBITEP modified GC electrode BAS (Bioanalytical Systems, West Lafayette, IN, USA) model MF-2012 were used as a working electrode in all electrochemical experiments such as CV, and SWAdSV.

### *2.2. Synthesis of 4-(2-((6-nitro-1H-benzo[d]imidazol-2-yl)thio)ethyl)phenol (NBITEP)*

A solution containing 2-mercapto-5-nitrobenzimidazole (1.0 mmol), 4-hydroxyphenethyl bromide (1.0 mmol), and $K_2CO_3$ (1.2 mmol) in acetone was refluxed at 40 °C for 2 hours. Following cooling, the solvent was evaporated to dryness. The resulting residue was then treated with 25 mL of water. The solidified product was filtered, washed with water, and subjected to recrystallization from ethanol, yielding the compounds as reported by Yurttaş et al. in 2014 and 2016 (Yurttaş et al., 2014; Yurttaş et al., 2016). The analysis results from FTIR and NMR (1H and 13C) are as follows: Yield: 76%, melting point: 150.5 °C. FTIR (ATR) $cm^{-1}$: 3373 (N–H), 3205 (O-H), 1612–1421 (C=C, C=N), 1512-1319 (NO2), 819 (1,4-disubstituted benzene). $^1$H-NMR (500 MHz, DMSO-d6): δ = 2.95 (2H, t, J=7.50 Hz, -CH2-), 3.52 (2H, t, J=7.50 CH2), 6.71 (2H, d, J=8.50 1,4-phenyl), 7.10 (2H, d, J=8.00 1,4-phenyl), 7.56 (1H, d, J=9.00 Hz, Benzimidazole-H4), 8.03 (1H, dd, J=2.50, J=8.50, Hz, Benzimidazole-H5), 8.29 (1H, d, J=9.00 Hz, Benzimidazole-H7), 9.26 (1H, s, OH), 13.29 (1H, s, Benzimidazole-NH). $^{13}$C-NMR (125 MHz, DMSO-d6): δ =33.18, 34.81, 110.59, 113.69, 115.61, 115.67, 117.38, 129.99, 130.45, 139.45, 141.97, 142.01, 156.36. HRMS (m/z): [M+H]+ calculated for C15H13N3O3S: 316.0750; found 316.0750 (Fig. 1).

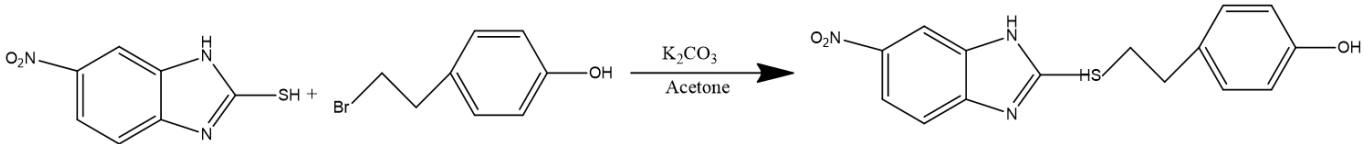


**Figure 1.** Synthesis mechanism of NBITEP molecule.

### *2.3. Preparation and polishing GC electrode surface*

The GC electrode underwent preparation for the experiments through a polishing process on micro cloth pads (Buehler, USA) to achieve a mirror-like appearance. This involved initial polishing with fine wet emery papers (grain size 4000), followed by polishing with 1.0 and 0.3 mm alumina slurry. Subsequently, the GC electrode underwent sonication in both water and a 1:1 (v/v) mixture of isopropyl alcohol (IPA) and $CH_3CN$ (IPA + $CH_3CN$) for 10 minutes, following a specific sequence (Mülazımoğlu et al., 2012; Mülazımoğlu et al., 2011).

## 3. Results and Discussion

The electrochemical characterization revealed distinctive features of NBITEP-modified electrode surfaces, indicating favorable adsorption and redox processes. The SWAdSV technique demonstrated high sensitivity and selectivity towards $Hg^{2+}$ ions, facilitated by the unique properties of NBITEP. Moreover, the study elucidated the underlying mechanisms governing the electrochemical behavior of NBITEP and its interaction with $Hg^{2+}$ ions, providing valuable insights into sensor design and optimization.

### *3.1. Modification and characterization of GC electrode surface*

As a step after synthesis processes and structure analysis, modification processes were carried out using the electrochemical CV technique. By using 1 mM NBITEP solution prepared in 100 mM $NBu_4BF_4$ solution dissolved in acetonitrile in anhydrous medium, the modification was made against the $Ag/Ag^+$ reference electrode in the range of 0.0 V to +2.3 V, at a scanning speed of 100 mV $s^{-1}$ and for 10 cycles. The voltammogram of the modification is given in Figure 2.

Upon examining the modification voltammogram in Figure 2A, it becomes apparent that the peaks vanish starting from the second cycle. This observation suggests the coverage of the electrode surface by the molecule. Despite initiating the modification process from the first cycle, conducting it through 10 cycles serves the purpose of avoiding the persistence of small openings, referred to as pinholes, on the surface. In this investigation, the characterization processes employed the CV technique in both aqueous and non-aqueous mediums.

The reduction of the nitro group in the molecule was carried out using CV in 100 mM HCl medium with a potential range from +0.2 V to -1.0 V, scanning speed of 100 mV $s^{-1}$ and with 10 cycles (Figure 2B). After modification and reduction, surface characterizations were made with CV using ferrocene in non-aqueous medium (Figure 3A) and ferricyanide redox probes in aqueous medium (Figure 3B).

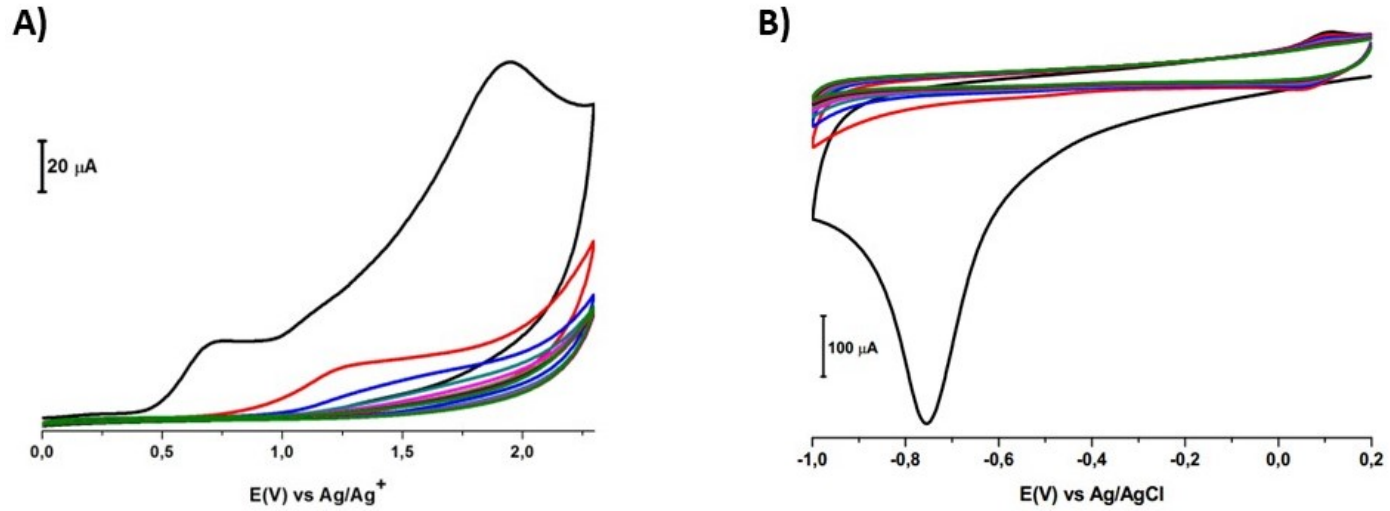


**Figure 2.** A) The modification voltammogram of NBITEP onto the GC surface was conducted within the potential range of 0.0 V to +2.7 V, employing a scanning rate of 100 mV s-1 with 10 cycles. B) Cyclic voltammogram illustrating the reduction of the nitro group to the amine group on the reduced NBITEP modified GC electrode surface in a 100 mM HCl solution medium.

Figure 2A illustrates the full modification of the NBITEP molecule on the surface of the GC electrode in an non-aqueous medium, while Figure 2B depicts the reduction of -$NO_2$ and -$NH_2$ groups on the reduced NBITEP modified GC electrode surface in an acidic medium.

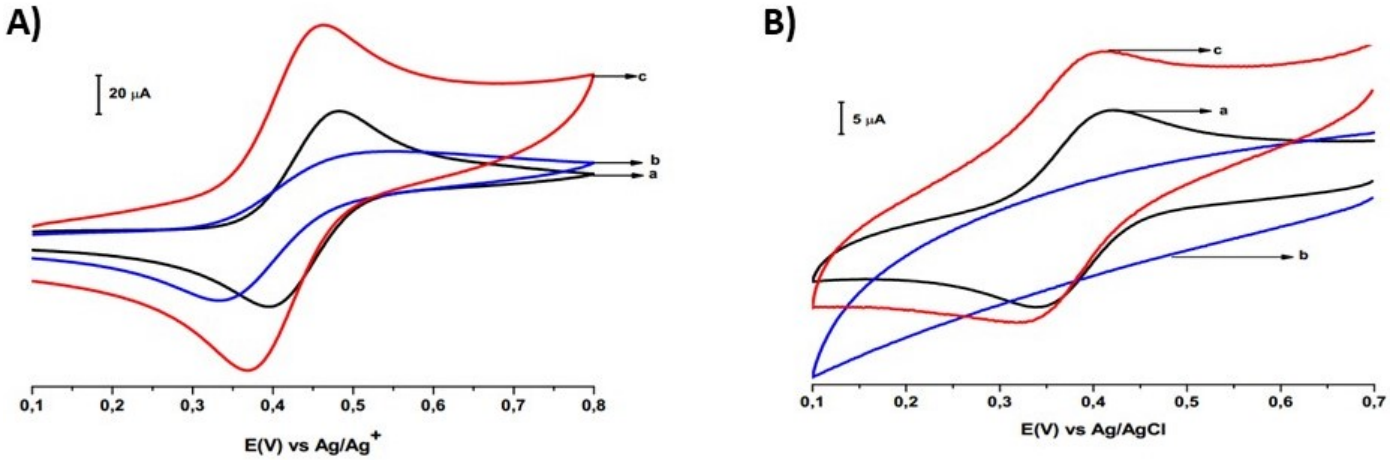


**Figure 3.** Comparative cyclic voltammograms are presented for: **A)** Ferrocene redox probe solution (1 mM) versus Ag/Ag+ (10 mM) in CH3CN with 100 mM NBu4BF4, employing a scanning rate of 100 mV s-1. **B)** Redox probe solution of Fe(CN)63- (1 mM) versus Ag/AgCl/3 M KCl reference electrode in BR buffer solution at pH 2.0, using a scanning rate of 100 mV s-1. The voltammograms are obtained on **a)** the bare glassy carbon (GC) electrode surface, **b)** the GC electrode surface modified with NBITEP, and **c)** the reduced NBITEP/GC electrode surface.

The characterization of the newly obtained surfaces after modification in an non-aqueous medium electrochemically and subsequent reduction in an acidic medium was performed by anodic scanning in the presence of ferrocene redox probe in the non-aqueous medium and cathodic scanning in the presence of ferricyanide redox probe in the aqueous medium. Figures 3A and 3B demonstrate that both in non-aqueous and aqueous media, NBITEP molecule is fully modified and reduction is fully realized. Specifically, while the Bare GC electrode surfaces are electroactive, they become electroinactive due to the presence of the functional group -$NO_2$ after NBITEP modification, and after reduction, they become electroactive again due to the formation of -$NH_2$ groups on the surface of the molecule.

### *3.2. Electrochemical reaction mechanism of oxidation and reduction of NBITEP on GC electrode surface*

After the establishment of NBITEP, the nitro groups can undergo electrochemical reduction to form amines, as illustrated in Figure 4. The cathodic sweep of the cyclic voltammogram recorded from a glassy carbon electrode modified with NBITEP exhibits an irreversible reduction peak at approximately −750 mV. The reduction peak area for the conversion of nitro to amine is comparatively smaller on carbon electrodes. In a 100 mM HCl medium, the nitro groups on the modified surface, following characterization procedures, were successfully reduced to amine groups (Mulazimoglu and Demir Mulazimoglu, 2012). Following this process, conducted within the potential range of 0.200 to −1.000 V at a sweep rate of 100 mV $s^{-1}$ and through 10 cycles (Figure 2B), the initially electroinactive surface became electroactive.

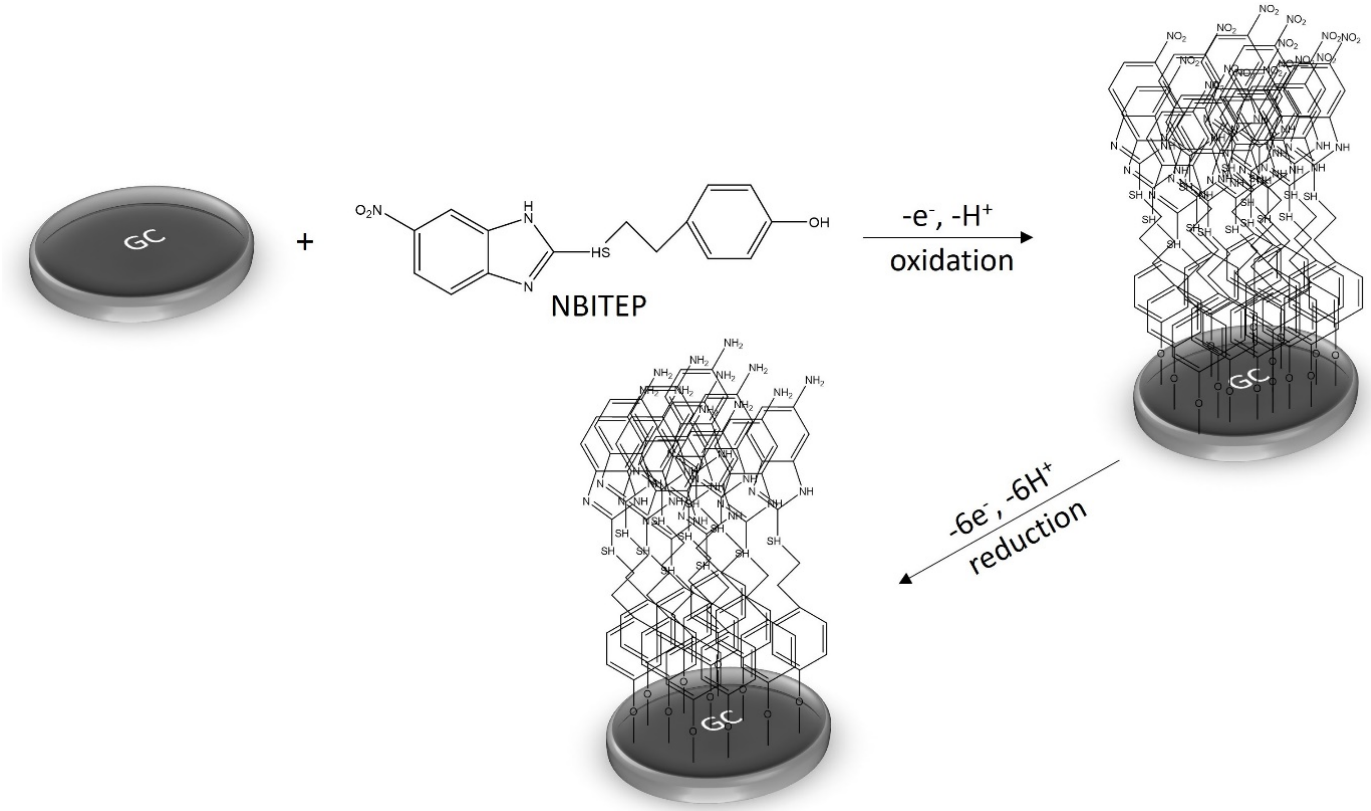


**Figure 4.** The process involves the modification of NBITEP onto the GC electrode surface, followed by elucidating the mechanism of reduction from the nitro group to the amine group on the surface of the modified electrode.

### *3.3. Determination of $Hg^{2+}$ onto the NBITEP/GC electrode surface*

In this part, first of all, the deposition time study was carried out using the SWAdSV technique (Fig. 5). As a result of this study, it was understood from the superimposed image of the voltammograms that the appropriate time for deposition was 360 seconds.

The overlayed image of the SWAdSV results obtained using solutions of $Hg^{2+}$ ions at different concentrations is given in Figure 6. Accordingly, it seems that $Hg^{2+}$ ion can be determined with the SWAdSV technique using the reduced NBITEP/GC sensor electrode in the concentration range of 1 mM to 1 µM.

For the conclusion and discussion section of the thesis, you can elaborate on the findings and implications of the electrochemical studies conducted on the NBITEP molecule, particularly regarding its potential application as a sensor for detecting $Hg^{2+}$ ions.

Begin by summarizing the key findings of the research. Highlight the successful synthesis and structural elucidation of the NBITEP molecule and its electrochemical behaviors observed through CV technique. Discuss the results obtained from the characterization processes, emphasizing any changes observed in the electrochemical properties of the GC electrode upon modification with NBITEP. This could include shifts in peak potentials, changes in peak currents, and alterations in voltammograms.

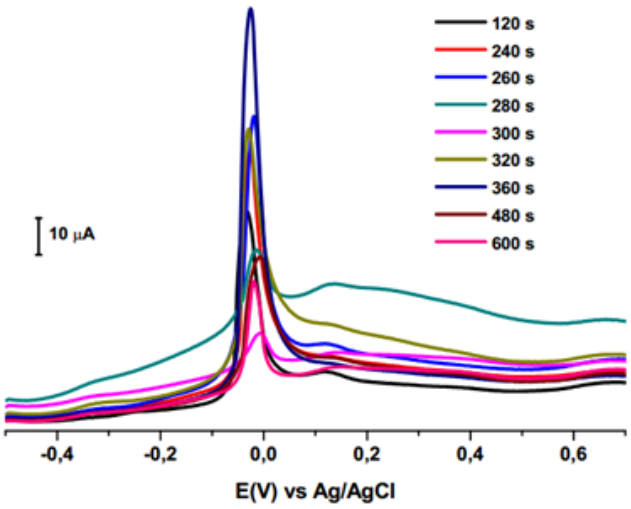


**Figure 5.** Voltammograms of 1 mM $Hg^{2+}$ using reduced NBITEP/GC electrode surface with applying different accumulation times, under the following conditions: PBS at pH 7.0, wave frequency 25 Hz, pulse size 50 mV and stirring rate 500 rpm, through employing SWAdSV technique.

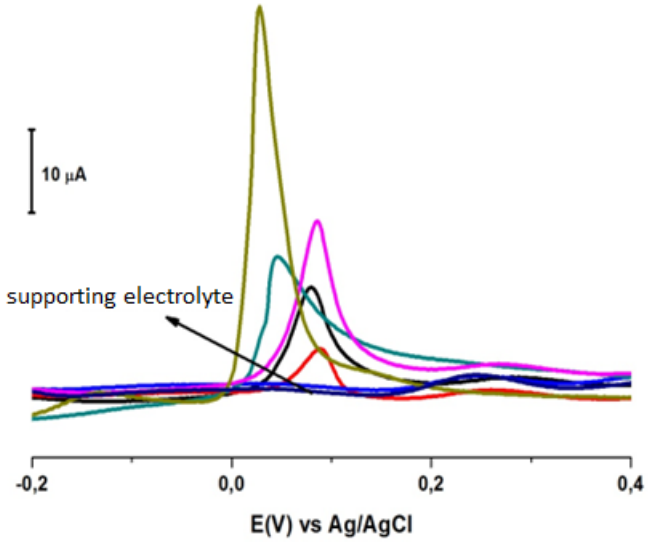


**Figure 6.** Voltammograms of different $Hg^{2+}$ ion concentrations ($1.10^{-3}$, $5.10^{-4}$, $1.10^{-4}$, $5.10^{-5}$, $1.10^{-5}$, $1.10^{-6}$ M) using reduced NBITEP/GC electrode surface in PBS at pH 7.0, under the following conditions: -0.2/+0.4 V potential range, accumulation time 360 s, wave frequency 25 Hz, pulse size 50 mV and stirring rate 500 rpm, through employing SWAdSV technique.

Present the results of the investigation into the potential of the reduced NBITEP modified GC electrode as a sensor for detecting $Hg^{2+}$ ions. Discuss the sensitivity, selectivity, and detection limits achieved using SWAdSV in a PBS medium. Compare the performance of the reduced NBITEP modified GC electrode with other existing methods for $Hg^{2+}$ ion detection. Highlight any advantages, such as enhanced sensitivity or lower detection limits, offered by the proposed sensor electrode. Provide insights into the underlying mechanisms governing the electrochemical behavior of NBITEP and its interaction with $Hg^{2+}$ ions at the electrode surface. Discuss any potential redox processes or adsorption phenomena involved in the detection mechanism. Discuss the broader implications of the research findings and potential applications of the reduced NBITEP modified GC electrode in environmental monitoring or analytical chemistry. Suggest future research directions, such as exploring the sensor's performance under different experimental conditions or investigating its applicability in real samples.

Summarize the main conclusions drawn from the study, emphasizing the significance of the findings in advancing the field of electrochemical sensing for mercury detection. By covering these points in the conclusion and discussion section, you can provide a comprehensive overview of the research outcomes and their implications for the scientific community.

## 4. Conclusions and Recommendations

The Master's Thesis outlined provides a comprehensive exploration of the NBITEP molecule, focusing on its synthesis, structural elucidation, and electrochemical behaviors. Through meticulous experimentation, the study utilized a GC electrode as the working electrode, employing CV for modification and characterization processes. Notably, the research delved into the potential of the modified surface to serve as a sensor electrode for detecting $Hg^{2+}$ ions, utilizing SWAdSV. The detailed examinations conducted in a PBS medium specifically targeted $Hg^{2+}$ ion detection, showcasing the efficacy of the reduced NBITEP modified GC electrode surface as a promising sensor electrode. This work not only advances our understanding of the electrochemical properties of NBITEP but also highlights its potential application in sensitive and selective detection of heavy metal ions, contributing to the field of analytical chemistry and environmental monitoring.

The study employed both alcohol oxidation and alternating voltammetry techniques, common in electrochemical analysis, for modification. In an acidic environment, the nitro group on the surface was effectively reduced to the amine group using alternating voltammetry, resulting in an electroactive surface.

Following the modification process, comprehensive characterization and stability studies were conducted. The reduced NBITEP/GC electrode surface obtained was then investigated for its applicability as a sensor electrode for various metals using square wave stripping voltammetry in a phosphate buffer solution. The findings suggested that the reduced NBITEP/GC electrode surface, derived from these studies, exhibited promising potential as a suitable sensor electrode for detecting $Hg^{2+}$ ions.

A successful and efficient determination of $Hg^{2+}$ ions on a disposable reduced NBITEP/GC electrode surface was achieved in a phosphate buffer solution using the SWAdSV technique. The generated $Hg^{2+}$ standard curve covered a range from 1.0 mM to 1.0 µM, demonstrating the feasibility of a simple, cost-effective, and rapid method for quantifying $Hg^{2+}$ ions.

In conclusion, this research highlights the promising prospects of NBITEP as a sensor electrode for $Hg^{2+}$ detection, leveraging the SWAdSV technique. The comprehensive understanding of its electrochemical properties and behaviors paves the way for the development of efficient and reliable sensing platforms for various environmental and analytical applications. Further exploration and refinement of NBITEP based GC sensors hold significant potential for addressing contemporary challenges in environmental monitoring and public health.

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**CRediT author statement**

LA: She took part in all experimental procedures for this study, writing and reading the manuscript.
ŞK: She took part in all experimental procedures for this study, writing and reading the manuscript.
YÖ: The synthesis and characterization of the modifier species used in electrode modification have been carried out.
İEM: He carried out the operations of directing, controlling and interpreting the results of the experiments for the study. He also carried out the final reading of the manuscript.

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