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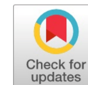
Overview of Analytical Methods for Characterizing the Charge Heterogeneity of Antibody-Drug Conjugates



Ayat Abbood

Abstract*: Antibody-drug conjugates (ADCs) are ideal candidates for selective therapy. ADCs display considerable heterogeneity resulting from various modifications. One of the molecular characteristics of ADCs is charge heterogeneity. Several analytical methods could be used to characterize the charge heterogeneity of ADC including ion exchange chromatography (IEC), isoelectric focusing (IEF), and capillary electrophoresis (CE). This work aimed to give insights into the different analytical techniques used to determine pI and the charge distribution profile of ADCs.

***Keywords*: Antibody, conjugates, charge, heterogeneity.**

I. INTRODUCTION

Cancer is the second leading cause of death worldwide. This dangerous disease is the focus of the pharmaceutical industry. Surgery and radiation therapy are often employed when the tumor is located in a certain tissue. In cases of metastasis, chemotherapy is used [1]-[5].

Most anti-cancer drugs have non-specific toxicity due to the lack of discrimination between healthy and tumor tissue. These cytotoxic drugs have a narrow therapeutic window, which limits their effectiveness and can lead to serious side effects. In addition, prolonged treatment can lead to tumors developing resistance to anticancer drugs. Consequently, it is necessary to aim cytotoxic drugs toward cancer cells [1]-[5].

Targeted prodrugs permit a more selective treatment. The ideal candidates for these prodrugs are antibody-drug conjugates (ADCs).

ADCs are monoclonal antibodies (mAbs) linked to cell-killing drugs. With high binding specificity for tumor-specific antigens, mAbs can target cell-killing payloads in tumor cells. Tumor-specific antigens, unique or overexpressed, can be found in various human tumor cells [1]-[5].

Certain mAbs can specifically recognize and bind to these tumor-associated antigens. These agents are effective for treating cancer by binding to specific antigens of cancer cells and inducing an immune response against targeted cancer cells [6]-[8].

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However, the effectiveness of treatment is often limited by the level of antibodies that cause cell death. While mAbs are highly discriminatory towards their targets, they can also be ineffective when used alone [9]-[14].

The lack of efficacy of most naked mAbs in cancer treatment can be overcome by adding radioactive isotopes or cytotoxic drugs to immunoglobulins, thus generating highly specific ADCs [15]-[20].

The manufacturing of therapeutic ADCs can be challenging due to the difficulty of demonstrating product consistency after changes in the process such as cell lines, manufacturing scale, conjugation reaction, production site, formulation, and purification process [1], [21]-[25].

ADC exhibits significant heterogeneity due to various modifications in the protein structure of the antibody itself, such as deamidation, amino acid substitution/deletion, differential glycosylation, glycation, etc. Additionally, the conjugation process by binding multiple drug molecules with antibodies increases ADC heterogeneity.

Such modifications can lead to the presence of various species in crude ADC preparations and final, purified products. Therefore, relevant methods should be used to characterize ADC.

Table I presents common analytical techniques for characterizing molecular heterogeneity or product-related impurities in mAbs [1].

As we can see in Table I, one of the molecular characteristics of ADC is charge heterogeneity. Several analytical methods can be used to characterize the charge heterogeneity of ADC including ion exchange chromatography (IEC), isoelectric focusing (IEF), and capillary electrophoresis (CE).

This study presents the different analytical techniques used to determine pI and the charge distribution profile of ADCs.

Table I: Analytical Techniques for Characterizing Molecular Heterogeneity or Product-Related Impurities in mAb or ADC Products

Molecular Characteristics	Analytical Technique
Purity and concentration determination	Size exclusion chromatography (SEC) SDS-PAGE (reduced and nonreduced) or CE-SDS, UV
Primary structure	Amino acid analysis, Mass spectrometry/peptide mapping N- and C-terminal sequencing

Secondary structure	Circular dichroism (CD) Infrared spectroscopy (IR)
Tertiary and higher-order structure	Size exclusion chromatography (SEC) Analytical ultracentrifugation (AUC) Field-flow fractionation (FFF) Direct binding and biological assays
Charge variants	Ion exchange chromatography (IEC) Isoelectric focusing (IEF-gel) Chromatofocusing
Glycosylation	Mass spectrometry NP/RP-HPLC
Chemical modification (oxidation, photon cross-linking, glycation, etc)	Mass spectrometry/peptide mapping SDS-PAGE (reduced and nonreduced) Ion exchange chromatography (IEC)

II. ANTIBODY MOLECULAR STRUCTURE

Antibodies, also known as immunoglobulins (Ig), are glycoproteins secreted by specialized B lymphocytes called plasma cells. They consist of four polypeptides: two identical copies of the heavy and the light chain linked together by disulfide and noncovalent bonds. The resulting molecule is often represented by a schematic Y-shaped molecule diagram (Figure 1).

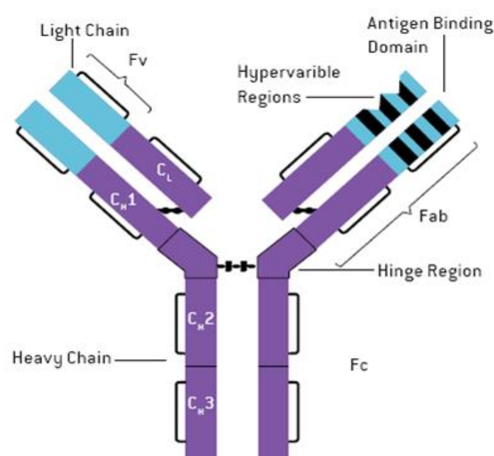

Figure 1: Structure of Monoclonal Antibody

Light chain (LC) consists of a variable region and a constant region of equivalent length. Similarly, the heavy chain (HC) is divided into variable and constant regions. However, the heavy chain has one variable and at least three constant zones.

The region of the antibody called the Fab (fragment antigen binding) consists of one constant and one variable domain for each heavy and light chain. The variable domain is the most important region for antigen binding. The hypervariable regions of the light chains and heavy chains are known as complementarity-determining regions (CDRs). They are responsible for binding to the antigen. The two arms (Fab) of the antibody molecule and the tail (Fc) or crystallizable part are connected by a region rich in proline, threonine, and serine, called the hinge [1].

Antibodies perform two essential roles:

Antibodies bind to an epitope on the antigen using Y arms. Each arm or monovalent antibody fragment (Fab) domain contains a binding site, making each antibody molecule at least bivalent.

The Fc domain of the Y imparts the antibody with biological effector functions such as natural killer cell activation, activation of the classical complement pathway, and phagocytosis.

mAbs are antibodies produced by a single clone of B cells. Unlike polyclonal antibodies, mAbs are monospecific and homogeneous, making them powerful tools for treatment development.

III. THERAPEUTIC MONOCLONAL ANTIBODY

Since the early 1980s, mAbs have moved from discovery to clinical practice. Many of these mAbs, alone or attached to payloads (such as chemical drugs, toxins, radionuclides, enzymes, or cytokines), are used to treat cancer [1]-[10].

The first mAb approved by the US Food and Drug Administration (FDA) for human use was the murine anti-CD3 mAb, muromonab (OKT3), used to treat organ transplant rejection. OKT3 provided the first lesson for the therapeutic mAbs, i.e. murine mAbs have a short half-life, strong immunogenicity, and suboptimal effector functions when used in humans. These problems have been solved by converting mouse mAbs into human protein formats by genetic engineering [11]-[25].

From 1984 to 1988, the chimerization and humanization of mAbs were carried out. A chimeric mAb is designed to contain the variable regions of the mouse antibody and the constant regions of the human antibody, whereas in the humanized mAb, only the complementary determining regions (CDRs) are of murine origin (Figure 2).

Fully human mAbs were created by phage display or by selecting variable regions of human origin. In addition, human immunoglobulin (Ig) transgenic mice are currently being used to produce fully human antibodies.

The first mAb approved to treat cancer, rituximab, a chimeric anti-human CD20 mAb, was approved in 1997. A decade later, eight more mAbs were approved for cancer treatment and hundreds of mAbs are undergoing preclinical and clinical evaluation.

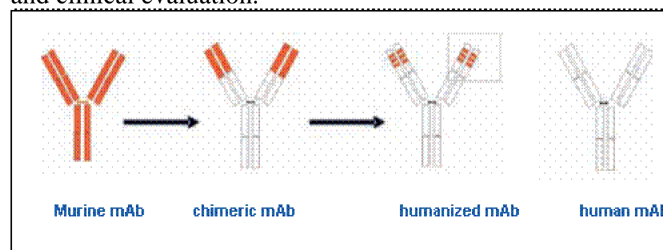

Figure 2: Structure of Murine, Chimeric, and Humanized mAb

IV. ANTIBODY-DRUG CONJUGATES (ADCS)

Naked mAbs have been used for the treatment of cancer. Indeed, since most naked mAbs are not effective enough in cancer treatment, different methods have been explored to improve their anticancer activity, including linking the antibodies to radioisotopes, chemotherapeutic drugs, or toxins (Figure 3).

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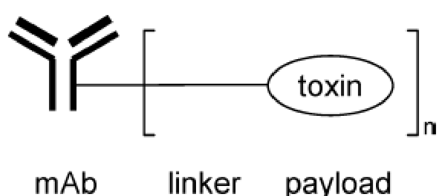

Figure 3: Schematic Representation of an Antibody-Drug Conjugate

The most developed of these is the radio-immunotherapy in which a radioisotope is conjugated to a mAb. Another treatment is based on a cytotoxic agent chemically linked to a monoclonal antibody that recognizes a tumor-associated antigen. mAbs preferentially deliver cytotoxic agents to tumor cells expressing the target antigen, thereby limiting systemic toxicity [20]-[25].

V. ANALYTICAL TECHNIQUES OF CHARGE CHARACTERIZATION

A. Chromatofocusing

Chromatofocusing allows the separation of proteins based on differences in their isoelectric point (pI), by creating a pH gradient through ion exchange stationary phases (cation exchangers CEX or anion exchangers AEX) [26]-[35].

To separate proteins according to their different pIs, a chromatofocusing medium is equilibrated with a starting buffer at a pH slightly higher than the highest pH required. The elution buffer (adjusted to the lowest pH required) passes through the column and begins the titration of the amines on the medium and the proteins. As the buffer flows through the column, the pH is lowered and a moving, decreasing pH gradient is generated (Figure 4) [27]-[28].

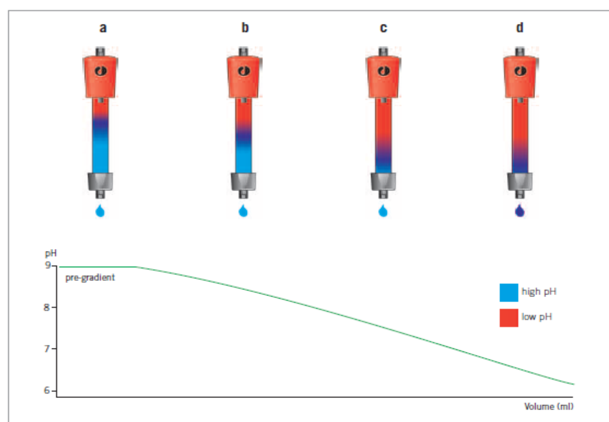

Figure 4: Development of a pH gradient in a Chromatofocusing Column. The column is Pre-Equilibrated With Start Buffer At High Ph (A) And Elution with Buffer At Low ph (b, c, d,) Generates A Descending Linear Ph Gradient

After a pre-gradient volume of elution buffer has passed, the sample (in the start buffer) is introduced into the positively charged column. The proteins in the sample are titrated (pH adjusted) immediately after being introduced into the column. Proteins in the sample become negatively charged at a pH above their pI. Thus, they are retained near the top of the column (bound to positively charged amino groups). Proteins, at a pH lower than their pI, will migrate down the column with the buffer flow. They will not bind until they reach a region with a pH higher than their pI (Figure 5) [29].

As the pH continues to decrease near the top of the column, any protein that falls below its pI becomes positively charged, is repelled by the positively charged amine groups, and begins to migrate down the column with the elution buffer, moving faster than the pH gradient is moving down the column. However, as the protein moves down the column, the pH of the medium increases. When the protein reaches a region with pH above its pI, it becomes negatively charged and re-associated with the column again. The protein remains bound until the developing pH gradient lowers the local pH. Once again, the protein falls below its pI, becomes positively charged, and migrates down the column, catching up to the gradient. This process continues until the protein is eluted from the column at a pH close to its pI (when it has almost no net charge). Proteins with different pIs move at different speeds through the column as the pH gradient develops, continually associating and dissociating. During the chromatofocusing, they are concentrated into narrow bands and finally eluted. The protein with the highest pI elutes first and the protein with the lowest pI is eluted last [29].

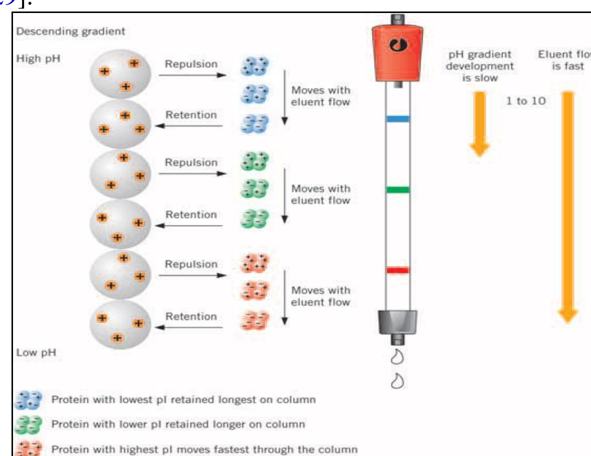

Figure 8: Proteins with Different pIs Separate as they Pass into the Column. Molecules with the Same Isoelectric Point are Focused in Narrow Bands During the Separation

A study was performed to characterize 10 model mAbs using the cation exchange pH gradient method [36]. The results demonstrated that retention and resolution can be modeled in cation exchange pH gradient mode.

A study was performed to evaluate the effects of some anion exchange chromatofocusing conditions on the characterization of the charge profile of a naked mAb and its drug conjugate [30]. Figure 9 shows chromatofocusing chromatograms of the naked mAb and its drug conjugates under the optimal chromatofocusing conditions. The naked mAb was less heterogeneous and acidic than the corresponding conjugate.

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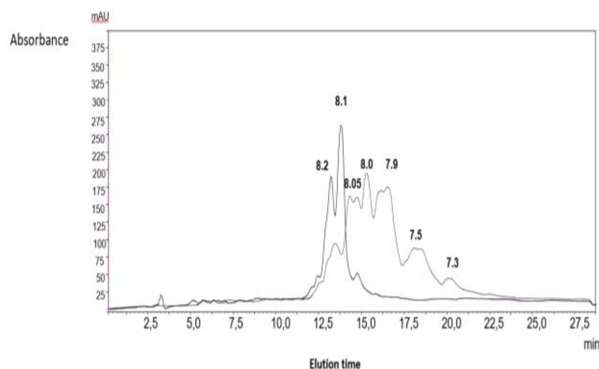

Figure 9: Chromatofocusing Profile of Naked mAb-1 and Drug- mAb-1 Conjugate

Research was performed to determine pI and relative charge variants of 23 therapeutic mAbs [37]. The results demonstrated that CEX conditions can be used to evaluate the relative amounts of mAb charge variant. Figure 10 shows the chromatograms of elotuzumab with generic and optimized gradient conditions.

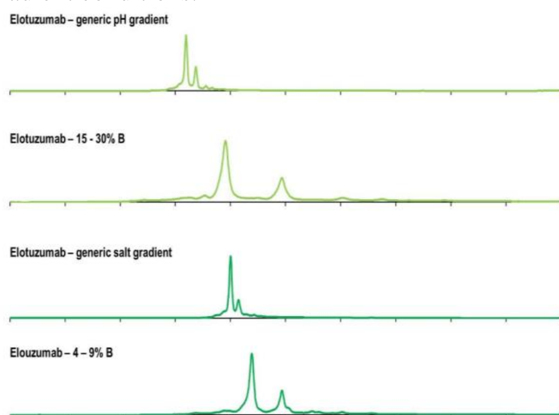

Figure 10: Comparison of Generic and Optimized Gradients in CEX Using Both pH And Salt Gradient Elution Modes of Charge Variants of Elotuzumab

B. Imaged Capillary Ief

Isoelectric focusing (IEF) is a high-resolution technique for separating proteins by their pI. It is commonly used for protein pI determination, identification, characterization, and stability monitoring. Compared to conventional gel IEF, cIEF offers higher resolution, speed, and quantitative analysis [38]-[58][59][60].

In capillary isoelectric focusing (cIEF), the capillary is filled with a solution of carrier ampholytes and sample. The anode end of the capillary is dipped into an acidic solution and the cathode end into a basic solution. When an electric field is applied, the presence of the ampholytes causes the creation of a pH gradient along the capillary. The charged components of the sample, such as proteins, then move into the capillary until they reach a pH region equal to their pI, where they become neutral. As a result, they stop migrating, leading to a series of narrowly focused zones. Conventional capillary isoelectric focusing (cIEF) is characterized by two steps: the focalization of analytes and the movement of the focused sample zone to the detection point (mobilization). The mobilization step can be achieved by applying a hydraulic force (pressure, vacuum, gravity). This step is also performed by adding ions. Indeed, the mobilization step greatly affects the focusing ability, separation efficiency, and method reproducibility. Additionally, because the single-point detection cannot trace the analyte after injection into the column or monitor the progress of the separation process, detailed insights about the separation mechanism, separation dynamics, sample adsorption, and band broadening inside the column are unknown. As a result, single-point detection is not an ideal detection method. Whole-column detection (WCD), for which there is no mobilization step, allows for simultaneous detection along the entire length of a column and is a better option [38]-[58].

Several reviews have discussed the state of CE-based methods for characterizing mAbs and ADCs. There are only a few published studies on the different factors affecting the separation of charge isoforms of mAbs and their ADCs. For example, one study aimed to develop an imaged cIEF method to characterize the charge heterogeneity of ADCs [42]. The studied factors included focusing time, sample composition, pH range, carrier ampholyte percentage, conjugated antibody concentration, and urea concentration. Other work aimed to evaluate the charge variant profile of antibody-tobramycin conjugates using an icIEF. Figure 11 shows the good separation of charge isoforms charge of the cleavable conjugates of two antibodies with tomaymycin [43].

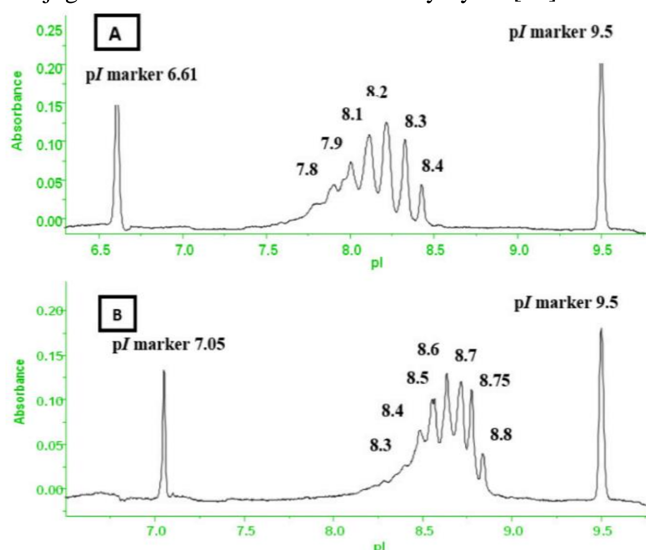

Figure 11. Analysis using icIEF of (a) Cleavable mAb-1 To Maymycin Conjugate, (b) Cleavable Mab-2 Tomaymycin Conjugate

Evaluation of charge variant profiles is one of the tools used to ensure the quality of mAbs and their ADCs. Several studies have compared the properties of icIEF with those of chromatofocusing in the separation of charge isoforms of mAbs and ADCs. For example, the charge variant profiles obtained by weak anion exchange chromatofocusing and icIEF methods for two unconjugated antibodies (mAb-1 and mAb-2) and their maytansine conjugates were compared. Significant differences were observed between the chromatofocusing and icIEF profiles for the mAbs and ADCs studied [36].

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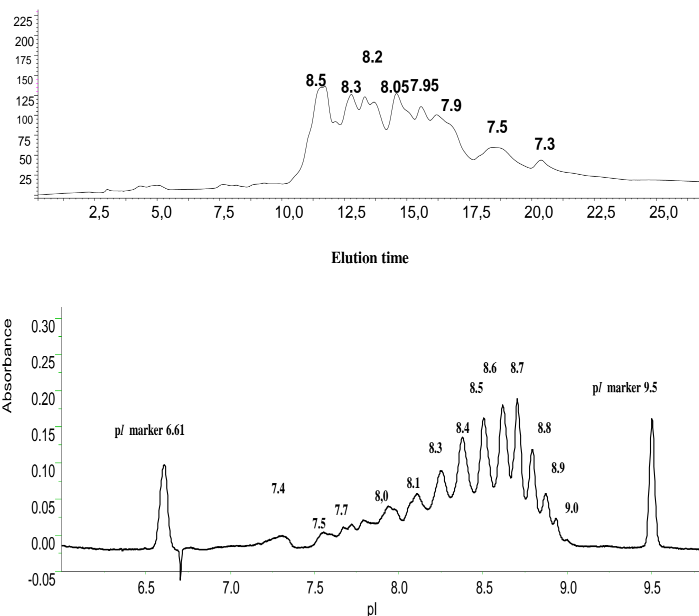

Figure 12: Chromatofocusing Profile of Conjugated mAb-2 (A): Ic IEF profile of Conjugated mAb-2 (B)

VI. CONCLUSION

Cancer is the second most common cause of death worldwide. One promising targeted therapy is antibody-drug conjugates. Antibody-drug conjugates have several isoforms distinguished by mass, charge, and pI. The quality of monoclonal antibodies and their drug conjugates are assessed using various analytical techniques. The charge heterogeneity profile ensures the quality of monoclonal antibodies and their drug conjugates. Chromatofocusing and imaged capillary isoelectric focusing methods are employed to achieve this goal. This review is dedicated to giving insights into the chromatofocusing and imaged capillary isoelectric focusing methods used to assess the charge heterogeneity profile of these compounds.

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Ethical Approval and Consent to Participate	No, the article does not require ethical approval and consent to participate with evidence.
Availability of Data and Material	Not relevant.
Authors Contributions	I am only the sole author of the article.

REFERENCES

1. Wagh A, Song H, Zeng M, Tao L, Das TK. Challenges and new frontiers in analytical characterization of antibody-drug conjugates. MAbs. 2018;10(2):222-243. doi:10.1080/19420862.2017.1412025
2. Chbani D, Abbood A, Alkhayer M. Determination of Nitrite and Nitrate Ions levels in some processed meats marketed locally. Research Journal of Pharmacy and Technology. 2018;11(4):1442-1447. doi: 10.5958/0974-360X.2018.00269.X.
3. Abbood A, Malek Z, Al-Homsh Y, Thallaj N. In vitro Study for Antibiotic resistance of bacteria causing Urinary Tract Infection from Syrian adults. Research Journal of Pharmacy and Technology. 2022;15(10):4727-2. doi: 10.52711/0974-360X.2022.00794. https://doi.org/10.52711/0974-360X.2022.00794
4. Isbera M, Abbood A, Ibrahim W. Weight and Content Uniformity of Warfarin Sodium Half Tablets. Research Journal of Pharmacy and Technology. 2016; 9(3):215-218. doi: 10.5958/0974-360X.2016.00039.1 https://doi.org/10.5958/0974-360X.2016.00039.1
5. Abbood A, Layka R. Weight and content uniformity Study of captopril half-tablets. Research Journal of Pharmacy and Technology. 2017;10(6):1621-1626. doi: 10.5958/0974-360X.2017.00285.2. https://doi.org/10.5958/0974-360X.2017.00285.2
6. Abbood A, Malek Z, Thallaj N. Antibiotic resistance of urinary tract pathogens in Syrian children. Research Journal of Pharmacy and Technology. 2022;15(11):4935-9. doi: 10.52711/0974-360X.2022.00829. https://doi.org/10.52711/0974-360X.2022.00829
7. Abbood A. Determination of phenolic content and antioxidant activity of some cosmetic creams available in the Syrian market. Journal of Chemical and Pharmaceutical Sciences. 2018;11:280-3. https://doi.org/10.30558/jchps.20181104006
8. Zrekah GH, Diab DA, Abboud AY. Determination of Protein and fat oxidation levels in imported infant formula available in Syria. International Journal of Pharmacy and Pharmaceutical Sciences. 2016;8:169-72.
9. Abbood A, Aldiab D, HPLC determination of caffeine in some beverages and pharmaceutical dosage forms available in the Syrian market, Journal of Chemical and Pharmaceutical Sciences 3 (10), 1174-1179
10. Abbood A, Herrenknecht C, Proczek G, Descroix S, Rodrigo J, Taverna M, Smadja C. Hexylacrylate-based mixed-mode monolith, a stationary phase for the nano-HPLC separation of structurally related enkephalins. Anal Bioanal Chem. 2011 Apr;400(2):459-68. doi: 10.1007/s00216-011-4762-4. https://doi.org/10.1007/s00216-011-4762-4
11. Asaad RA, Abdullah SS. Breast Cancer Subtypes (BCSs) Classification according to Hormone Receptor Status: Identification of Patients at High Risk in Jableh- Syria. Research J. Pharm. and Tech. 2018; 11(8): 3703-3710. doi: 10.5958/0974-360X.2018.00680.7. https://doi.org/10.5958/0974-360X.2018.00680.7
12. Asaad RA. Hormone Receptor Status and its Relation to C-Reactive Protein and other Prognostic factors in Breast Cancer in Jableh- Syria. Research J. Pharm. and Tech. 2017; 10(9): 3003-3010. doi: 10.5958/0974-360X.2017.00532.7. https://doi.org/10.5958/0974-360X.2017.00532.7
13. Morkus R, Abbood A. A Survey of the Awareness and Practices of Antibiotic Use Among College Undergraduates and Graduates in Latakia. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) ISSN: 2582-7618 (Online), Volume-4 Issue-3, April 2024. DOI:10.54105/ijapsr.C4039.04030424. https://doi.org/10.54105/ijapsr.C4039.04030424
14. Machkour A, Thallaj NK, Benhamou L, Lachkar M, Mandon D. he Coordination Chemistry of FeCl3 and FeCl2 to Bis [2-(2, 3-dihydroxyphenyl)-6-pyridylmethyl](2-pyridylmethyl) amine: Access to a Diiron (iii) Compound with an Unusual Pentagonal-Bipyramidal/Square-Pyramidal EnvironmentChemistry–A European Journal. 2006 :25;12(25): 6660-6668. https://doi.org/10.1002/chem.200600276. https://doi.org/10.1002/chem.200600276
15. Labban L, Thallaj N. The Effect of Magnesium Supplementation on Hba1c Level and Lipid Profile Among Type 2 Diabetics. Acta Scientific Nutritional Health, 2019, 3,10, 7-12. https://doi.org/10.31080/ASNH.2019.03.0435
16. Labban L, Thallaj N, Malek Z. The implications of E-cigarettes or" vaping" on the nutritional status. Journal of Medical Research and Health Sciences, 2019, 2, 11, 784-787. https://doi.org/10.15520/jmrhs.v2i11.128.
17. Labban L, Thallaj N, Labban A. Assessing the Level of Awareness and Knowledge of COVID-19 Pandemic among Syrians. Archives of Medicine, 2020, 12, 2:8, 1-5. DOI: 10.36648/1989-5216.12.2.309. https://doi.org/10.36648/1989-5216.12.3.309
18. Labban L, Thallaj N. The medicinal and pharmacological properties of Damascene Rose (Rosa damascena): A review. International Journal of Herbal Medicine,2020, 8, 2, 33-37. Corpus ID: 226058951.

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19. Besher S, Alallan L, Hasan Agha MI, Alshamaa I, Thallaj N. Influence of Soil Salinity on the Chemical Composition of Essential Oil of Rosmarinus officinalis in Syria. Research Journal of Pharmacy and Technology. 2024; 17(5):2282-8. doi: 10.52711/0974-360X.2024.00358. https://doi.org/10.52711/0974-360X.2024.00358
20. Khatib O, Alshimale T, Alsaadi A, Thallaj N. The Global Impact of HIV: A Comprehensive Review. IJAPSR, vol. 4, no. 3, pp. 6–19, Apr. 2024, doi: 10.54105/ijapsr.C4040.04030424. https://doi.org/10.54105/ijapsr.C4040.04030424
21. Thallaj N. Quick Review of Chemistry Related to the [Fe]-Hydrogenases. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2022. 2,4, 1-15. DOI:10.54105/ijapsr.C4016.062422. https://doi.org/10.54105/ijapsr.C4016.062422
22. Thallaj N. A Short Review of Some Examples of the Binding of Fullerenes C60 to Transition Metal Complexes. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2022. 2,6, 1-12. DOI: 10.54105/ijapsr.C4015.102622. https://doi.org/10.54105/ijapsr.C4015.102622
23. Thallaj N. Review of a Few Selected Examples of Intermolecular Dioxygenases Involving Molecular Oxygen and Non-Heme Iron Proteins. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2023. 3, 2, 1-18. DOI:10.54105/ijapsr.C4011.023223. https://doi.org/10.54105/ijapsr.C4011.023223
24. Thallaj N. A Brief Overview of the General Characteristics and Reactivity Towards Dioxygen of the Ferrous Tris (2-Pyridylmethyl Amine) Series Complexes is Presented. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2023. 3, 3, 1-18. DOI:10.54105/ijapsr.C4012.043323. https://doi.org/10.54105/ijapsr.C4012.043323
25. Thallaj N. Detecting Antioxidant Behavior for Phenolic Content of Some Beauty Care Creams in Syrian Market. Indian Journal of Advanced Chemistry, vol. 2, no. 1, pp. 10–14, Jan. 2024, doi: 10.54105/ijac.C2013.041322. https://doi.org/10.54105/ijac.C2013.041322
26. Baek J, Schwahn AB, Lin S, et al. New Insights into the Chromatography Mechanisms of Ion-Exchange Charge Variant Analysis: Dispelling Myths and Providing Guidance for Robust Method Optimization. Anal Chem. 2020;92(19):13411-13419. doi:10.1021/acs.analchem.0c02775. https://doi.org/10.1021/acs.analchem.0c02775
27. Goyon A, Excoffier M, Janin-Bussat MC, et al. Determination of isoelectric points and relative charge variants of 23 therapeutic monoclonal antibodies. J Chromatogr B Analyt Technol Biomed Life Sci. 2017;1065-1066:119-128. doi:10.1016/j.jchromb.2017.09.033. https://doi.org/10.1016/j.jchromb.2017.09.033
28. Fekete S, Beck A, Fekete J, Guillarme D. Method development for monoclonal antibody charge variants in cation exchange chromatography, Part II: pH gradient approach. J Pharm Biomed Anal. 2015;102:282-289. doi:10.1016/j.jpba.2014.09.032. https://doi.org/10.1016/j.jpba.2014.09.032
29. Li CM, William Hutchens T. Chromatofocusing. Methods Mol Biol. 1992;11:237-248. doi:10.1385/0-89603-213-2:237. https://doi.org/10.1385/0-89603-213-2:237
30. Kang X, Frey DD. High-performance cation-exchange chromatofocusing of proteins. J Chromatogr A. 2003;991(1):117-128. doi:10.1016/s0021-9673(03)00210-3. https://doi.org/10.1016/S0021-9673(03)00210-3
31. Kang X, Frey DD. Chromatofocusing of peptides and proteins using linear pH gradients formed on strong ion-exchange adsorbents. Biotechnol. Bioeng. 2004;87(3):376-387. doi:10.1002/bit.20122 https://doi.org/10.1002/bit.20122
32. 1 Nordborg A, Zhang B, He XZ, Hilder EF, Haddad PR. Characterization of monoclonal antibodies using polymeric cation exchange monoliths. J Sep Sci. 2009;32(15-16):2668-2673. doi:10.1002/jssc.200900283. https://doi.org/10.1002/jssc.200900283
33. Zhang L, Patapoff T, Farnan D, Zhang B. Improving pH gradient cation-exchange chromatography of monoclonal antibodies by controlling ionic strength. J Chromatogr A. 2013;1272:56-64. doi:10.1016/j.chroma.2012.11.060. https://doi.org/10.1016/j.chroma.2012.11.060
34. Sluyterman LAAE, Elgersma O. Chromatofocusing: Isoelectric focusing on ion-exchange columns. I. General principles. Journal of Chromatography A. 1978;150:17-30. https://doi.org/10.1016/S0021-9673(01)92092-8. https://doi.org/10.1016/S0021-9673(01)92092-8
35. Sluyterman LAAE, Wijdenes J. Chromatofocusing: Isoelectric focusing on ion-exchange columns. II. Experimental verification. Journal of Chromatogry A, 1978;150:1-44. https://doi.org/10.1016/S0021-9673(01)92092-8. https://doi.org/10.1016/S0021-9673(01)92092-8
36. Thallaj N, Abbood A. Charge heterogeneity characterization of naked and drug conjugated monoclonal antibody by anion-exchange chromatofocusing method. Arab Journal of Pharmaceutical Sciences. 7: 1: (2023): 79-87.
37. Goyon A, Excoffier M, Janin-Bussat MC, et al. Determination of isoelectric points and relative charge variants of 23 therapeutic monoclonal antibodies. J Chromatogr B Analyt Technol Biomed Life Sci. 2017;1065-1066:119-128. doi:10.1016/j.jchromb.2017.09.033. https://doi.org/10.1016/j.jchromb.2017.09.033
38. Ascione A, Belfiore M, Vesterinen J, Buda M, Holtkamp W, Luciani F. Charge heterogeneity of therapeutic monoclonal antibodies by different cIEF systems: views on the current situation. MAbs. 2024;16(1):2313737. doi:10.1080/19420862.2024.2313737. https://doi.org/10.1080/19420862.2024.2313737
39. Lechner A, Giorgetti J, Gahoual R, Beck A, Leize-Wagner E, François YN. Insights from capillary electrophoresis for characterization of monoclonal antibodies and antibody-drug conjugates. J Chromatogr B Analyt Technol Biomed Life Sci. 2019;1122-1123:1-17. doi:10.1016/j.jchromb.2019.05.014. https://doi.org/10.1016/j.jchromb.2019.05.014
40. Wu J, McElroy W, Haitjema C, Lück C, Heger C. Platform Methods to Characterize the Charge Heterogeneity of Three Common Protein Therapeutics by Imaged Capillary Isoelectric Focusing. Methods Mol Biol. 2021;2261:93-103. doi:10.1007/978-1-0716-1186-9_8. https://doi.org/10.1007/978-1-0716-1186-9_8
41. Ning W, Zhao Y. Characterization of ADCs by Capillary Electrophoresis. Methods Mol Biol. 2020;2078:251-262. doi:10.1007/978-1-4939-9929-3_17. https://doi.org/10.1007/978-1-4939-9929-3_17
42. Abbood A, Optimization of the Imaged cIEF Method for Monitoring the Charge Heterogeneity of Antibody-Maytansine Conjugate, Journal of Analytical Methods in Chemistry, 2023, Article ID 8150143, 10 pages. https://doi.org/10.1155/2023/8150143. https://doi.org/10.1155/2023/8150143
43. Abbood A, Monitoring the charge variant profile of antibody-tomaymycin conjugates by icIEF method, Acta Pharm. Sci. 2023, 62 (1), 226-239. https://doi.org/10.23893/1307-2080.APS6215
44. Labban L, Thallaj N, 2019. Acta Scient. Nutr. Health, 3: 7-12. https://doi.org/10.36648/1989-5216.12.3.309
45. Thallaj NK, Przybilla J, Welter R, Mandon D. A ferrous center as a site for a nitrile group into a carboxamide in mild conditions. J. Am. Chem. Soc. 2008, 130, 2414-2415. https://doi.org/10.1021/ja710560g. https://doi.org/10.1021/ja710560g
46. Thallaj N. Microwave-Assisted Synthesis of Oxadiazole and Thiazolidine Derivatives. Indian Journal of Advanced Chemistry, 1, 3, 2022. 10-14. DOI:10.54105/ijac.d2015.102222. https://doi.org/10.54105/ijac.D2015.102222
47. Thallaj N. Synthesis of a New Ligand Tris (2-pyridyl methyl) amine functionalized by a methoxy group and study of Dichloroferrous complexes, its reactivity to dioxygen both in the presence and absence of substrate. International journal of applied chemistry and biological sciences 2021, 2 (4), 65-77.
48. Thallaj N. Efficiency in transporting molecular oxygen to iron(II) complexes with ligands type tri (2-pyridylmethyl) amine substitution aromatic in (α) position by a mechanism that mimics biological oxidation. International Journal of Research Publication and Reviews, 2021, 2, 10, 951-959.
49. Thallaj NK, Mandon D, White KA. The Design of Metal Chelates with a Biologically Related Redox-Active Part: Conjugation of Riboflavin to Bis (2-pyridylmethyl) amine Ligand and Preparation of a Ferric Complex Eur. J. of Inorg. Chem., 2007, 44–47. https://doi.org/10.1002/ejic.200600789. https://doi.org/10.1002/ejic.200600789
50. Thallaj NK, Orain PY, Thibon A, Sandroni M, Welter R, Mandon D. Steric Congestion at, and Proximity to, a Ferrous Center Leads to Hydration of α-Nitrile Substituents Forming Coordinated Carboxamides. Inorg Chem. 2014 Aug 4;53(15):7824-36. P7826-7827-7828. https://doi.org/10.1021/ic500096h. https://doi.org/10.1021/ic500096h
51. Thallaj NK, Rotthaus O, Benhamou L, Humbert N, Elhabiri M, Lachkar M, Welter R, Albrecht-Gary AM, Mandon D. Chemistry.

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2008;14(22):6742-53.P6745-6746-6747. https://doi.org/10.1002/chem.200701967

52. Thallaj N, Machkour A, Mandon D, Welter R. Square pyramidal geometry around the metal and tridentate coordination mode of the tripod in the [6-(3′-cyanophenyl)-2-pyridylmethyl] bis (2-pyridylmethyl) amine $FeCl_2$ complex: a solid-state effect. New. J. Chem., 2005, 29, 1555 – 1558. https://doi.org/10.1039/B512108F. https://doi.org/10.1039/b512108f
53. Wane A, Thallaj NK, Mandon D. The Reactivity of Molecular Dioxygen on a Series of Isostructural Dichloroferrous Complexes with Tripodal Tetraamine Ligands: General Access to μ-oxo Diferric Complexes, and Effect of α-Fluorination on the Kinetics of the Reaction. Chemistry A European Journal 14 (22), 6742-6753. https://doi.org/10.1002/chem.200701967 https://doi.org/10.1002/chem.200701967
54. Malek ZS, Labban LM. Photoperiod regulates the daily profiles of tryptophan hydroxylase-2 gene expression in the raphe nuclei of rats. International Journal of Neuroscience, 2021,131 (12), 1155-1161. https://doi.org/10.1080/00207454.2020.1782903
55. Abbood A, Thallaj N. Comparison between chromatofocusing and icIEF charge variant profiles of unconjugated monoclonal antibodies and their drug conjugates. Arab Journal of Pharmaceutical Sciences. 2023:7;(1).
56. Thallaj N. Characterization of charge heterogeneity of antibody-drug conjugate by anion-exchange chromatofocusing. Tishreen University Journal-Medical Sciences Series. (2023). 44,(6),21-29.
57. Salloum R, Baddour F, Abbood A. A Questionnaire to Evaluate Undergraduate Students' Consumption and Awareness of Non-Steroidal Anti-Inflammatory Drugs in Syria. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR), Volume-4 Issue-4, June 2024, pages 1-6. DOI: 10.54105/ijapsr.C4041.04040624. https://doi.org/10.54105/ijapsr.C4041.04040624
58. Zanboua R, Abbood A. Survey of Knowledge About the Interaction Between Food and Drugs Among the Syrian Population. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR), Volume-4 Issue-4, June 2024, pages 22-28. DOI: 10.54105/ijapsr.D4044.04040624. https://doi.org/10.54105/ijapsr.D4044.04040624
59. Park, H. (2022). The Simplest Pharmacokinetic Equation in ADC (Antibody Drug Conjugate) or PDC (Peptide Drug Conjugate) Research. In International Journal of Advanced Pharmaceutical Sciences and Research (Vol. 2, Issue 5, pp. 1–4). https://doi.org/10.54105/ijapsr.c4017.082522 https://doi.org/10.54105/ijapsr.C4017.082522
60. Sharif, Z. M., Kamal, A. F., & Jalil, N. J. (2019). Chemical Composition of Melaleuca Cajuputi Powell. In International Journal of Engineering and Advanced Technology (Vol. 9, Issue 1, pp. 3479–3483). https://doi.org/10.35940/ijeat.a2668.109119

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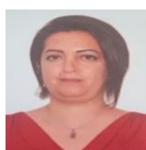

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