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Short Communication

Applications and Advancements of Molar Absorptivity in Clinical Biochemistry

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ABSTRACT

Molar absorptivity plays a crucial role in clinical biochemistry assays, offering an alternative or complementary approach to standard curve calibration in spectrophotometry. This short communication explores its applications in enzymatic and colorimetric assays, facilitating the direct quantification of biomolecules such as NADH, glucose, cholesterol, and various enzymes. Advances in computational methods, including machine learning, have enabled the prediction of molar absorption coefficients, potentially enhancing detection strategies. Additionally, biosensors and optical detection methods leverage molar absorptivity for improved quantification in point-of-care testing (POCT). However, ensuring analytical accuracy requires rigorous standardization and validation of molar absorptivity values. Future research should focus on refining computational models and establishing standardized protocols to enhance clinical applicability.

KEYWORDS

• Molar Absorptivity • Spectrophotometry • Enzymatic Assays • Machine Learning • Point-of-care Testing (POCT) • Biosensors • Optical Ketection • Standardization • Clinical biochemistry • Molar extinction coefficient

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INTRODUCTION

This short communication explores the utilization of molar absorptivity in clinical biochemistry assays, highlighting its role as an alternative or complement to traditional standard curve calibration in spectrophotometry. Spectrophotometers are widely used in analytical chemistry, operating based on Beer-Lambert's Law, which describes the relationship between absorbance and concentration. Molar Absorptivity is calculated by the following formula:

$A=\varepsilon cl$

Where,

A= Absorbance

ε= molar absorption coefficient (M^{-1} cm^{-1})

C= concentration (moles/L)

l= pathlength (cm) (1)

The interaction of light with a sample quantifies the amount of light absorbed or transmitted at specific wavelengths, facilitating the measurement of enzymes, metabolites, proteins, and drugs.

In cases where primary standards are unavailable for enzymatic assays, secondary standards or calibration factors are often used based on the molar extinction coefficients of reaction products.[1] This approach enables the direct quantification of biomolecules in various clinical applications.

Machine Learning for Molar Absorptivity Prediction

Advancements in computational methods, such as machine learning, have enabled the accurate prediction of molar absorption coefficients for thousands of compounds. Ksenofontov *et al*. demonstrated how machine learning models can predict the molar absorption of dye molecules like BODIPY (4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene), offering an alternative detection strategy.[2] However, these predicted values must undergo rigorous experimental validation to ensure their accuracy and applicability in clinical settings.

Applications of Molar Absorptivity in Clinical Chemistry

Enzyme Quantification Using NADH, enzymes are quantitated by measuring the increase or decrease of NADH (Nicotinamide adenine dinucleotide + Hydrogen) absorbance at 340 nm, the molar absorptivity (6.22 103 mol/L) of NADH is used to calculate enzyme activity. Similarly, other biomolecules can be quantified coupled with NADH.[1] Expanding the scope: key clinicalanalytes beyond NADH-based quantifications, molar absorptivity is employed in various enzymatic and colorimetric assays with analytes like glucose and cholesterol. Alanine Aminotransferase (ALT) & Aspartate Aminotransferase (AST): Enzymatic assays rely on chromogenic substrates, facilitating spectrophotometric quantification.[3]

MDA-TBA (Malondialdehyde-Thio barbituric Acid) Adducts: The concentration of MDA can be determined without a standard curve by measuring absorbance at 532 nm using its molar absorptivity. Biotinidase Assay: Biotinidase hydrolyzes biotinidyl-4-aminobenzoic acid (B-PABA), releasing p-aminobenzoic acid (PABA), which forms a purple dye upon diazotization.[5] Alkaline Phosphatase (ALP): No reagent calibration is needed, as ALP activity is directly calculated using the molar absorptivity of p-nitrophenyl phosphate.[6]

The clinical significance of POCT is being amplified by sophisticated quantification methods, which enhance the accuracy, reliability, and applicability of these tests in various healthcare settings.

Biosensors and Optical Detection Methods

Molar absorptivity plays a fundamental role in biosensors, particularly those utilizing optical detection. Many point-of-care testing (POCT) devices leverage optical methods such as absorption and fluorescence. ImageJ software, deep learning-powered paper-based sensors, and miniaturized infrared spectrometers have further enhanced the quantification of biomolecules.[7]

Standardization and Validation of Molar Absorptivity Values

Ensuring the accuracy and reliability of analytical results requires careful validation of molar absorptivity values. Certified Reference Materials (CRMs) provide the most reliable values with stated uncertainties. However, values provided by reagent manufacturers must be independently verified. While machine learning and computational methods can predict molar absorptivity values, these must be experimentally validated to maintain analytical precision.

However, it is essential to critically evaluate the quality of the research and the methods used to determine the values. Certified reference materials (CRMs) may provide molar absorptivity values with stated uncertainties. These are the most reliable sources. Reagent manufacturers may provide molar absorptivity values for their products. However, it is important to verify these values independently. Machine learning and other computational methods are increasingly used to predict molar absorptivity. However, these predicted values require experimental validation.

CONCLUSION

This study underscores the diverse applications of molar absorptivity in clinical biochemistry, spanning from traditional enzyme assays to modern point-of-care testing (POCT) biosensors. Proven protocols for bilirubin, biotinidase, and NADH-linked assays demonstrate that expanding this cost-effective approach to other parameters can streamline diagnostics and reduce healthcare expenses. The integration of machine learning for predicting molar absorption coefficients presents an exciting avenue for rapid and economical detection strategies. However, the standardization and experimental validation of molar absorptivity values remain critical to ensuring the accuracy and reproducibility of biochemical measurements. Future research should focus on developing standardized protocols and validating computational predictions to enhance clinical implementation.

REFERENCES

1. Bishop M.L., Fody E.P. Clinical chemistry: techniques, principles, correlations. Wolters Kluwer Health/Lippincott Williams & Wilkins; 2010.
2. Ksenofontov A.A., Lukanov M.M., Bocharov P.S. Can machine learning methods accurately predict the molar absorption coefficient of different classes of dyes? Spectrochim Acta A Mol BiomolSpectrosc. 2022 Oct 15; 279:121442. doi: 10.1016/j.saa.2022.121442
3. Resmi P.E., Sachin Kumar S., Alageswari D., Suneesh P.V., Ramachandran T., Nair B.G., Satheesh Babu T.G. Development of a paper-based analytical device for the colorimetric detection of alaninetransaminase and the application of deep learning for image analysis. Anal Chim Acta. 2021 Dec 15; 1188:339158. doi: 10.1016/j.aca.2021.339158. Epub 2021 Oct 14. PMID: 34794561.
4. Buege J.A., Aust S.D. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52:302-10. doi: 10.1016/s0076-6879(78)52032-6. PMID: 672633
5. Mohan I.K., Baba K.S., Prasad C., Hussain T., Alrokayan S.A., Naushad S.M., Akella R.R. A micro-method for biotinidase estimation using dried blood spots and comparison with plasma biotinidase. IOSR J Dental Med Sci. 2017; 16(06): 23-6.
6. Burtis C.A., Seibert L.E., Baird M.A., Sampson E.J. Temperature dependence of the absorbance of alkaline solutions of 4-nitrophenyl phosphate--a potential source of error in the measurement of alkaline phosphatase activity. Clinical chemistry. 1977 Sep 1; 23(9): 1541-7.
7. Nagaraja P., Avinash K., Shivakumar A., Krishna H. Quantification of creatinine in biological samples based on the pseudoenzyme activity of copper-creatinine complex. Spectrochim Acta A Mol BiomolSpectrosc. 2012 Jun 15; 92: 318-24. doi: 10.1016/j.saa.2012.02.104.
8. Yoon J.Y., Yoon J.Y. Spectrophotometry and optical biosensor. Introduction to Biosensors: From Electric Circuits to Immunosensors. 2013: 121-39.
9. Playfer J.R., Eze L.C., Bullen M.F., Evans D.A. Genetic polymorphism and interethnic variability of plasma paroxonase activity. Journal of medical genetics. 1976 Oct 1; 13(5): 337-42.