OPEN ACCESS

Kashf Journal of Multidisciplinary Research
Vol: 02 - Issue 1 (2025)
P-ISSN: 3007-1992 E-ISSN: 3007-200X
https://kjmr.com.pk

ADVANCING VITAMIN DETECTION: BENZIMIDAZOLIUM SALTS AS NEXT-GENERATION SPECTROPHOTOMETRIC MODIFIERS

Sheraz Tariq
Faculty of Sciences,
The Superior University Lahore, Pakistan.

Muhammad Atif
Faculty of Sciences,
The Superior University Lahore, Pakistan.

Muhammad Zeeshan Liaqat
Faculty of Sciences,
The Superior University Lahore, Pakistan.

Maryam Aslam
Department of Chemistry.
GC Women University Faisalabad.

**Corresponding author:* muhammad.atif.fsd@superior.edu.pk

Article Info

Abstract

Quantitative determination of vitamins such as vitamin C (ascorbic acid), vitamin B6 (pyridoxine) and vitamin E (α-tocopherol) find widespread use in pharmaceutical quality control, clinical diagnostics and nutritional studies. This purpose is often carried out using UV-Visible spectrophotometry, but much low molar absorptivity leads to poor sensitivity and high detection limits with this technique. The additional complexity of complex sample matrices, including interference and masking of analyte signals magnify these challenges. With regard to this, this paper describes the use of benzimidazolium salts as chemical modifier for improvement of spectrophotometric analysis of vitamins. The synthesized benzimidazolium salts, synthesized by methylation of benzimidazole with methyl iodide, associate with vitamins via formation of hydrogen bonds, π-π stacking and charge transfer, and enhance their optical properties. Structural integrity of the synthesized salts was also confirmed by FTIR and NMR spectroscopy. Substantial absorbance enhancements were observed upon incorporation of benzimidazolium salts into vitamin solutions. The values of increases in vitamins C 35%, B6 23% and E 42% were obtained. By such enhancement molar absorptivity improvements and detection and quantification limits improved by 20–30%, and trace level detection with better precision is allowed. The salts stabilize the excited states of vitamins and reduce matrix interferences to allow for stronger and more accurate analysis in complex matrices; mechanistic studies were performed. The modified spectrophotometric method was proven to be highly efficient and reproducible, and could be useful in pharmaceutical quality assurance and nutraceutical applications. In short, benzimidazolium salts are transformative in spectrophotometric analysis, suggesting a cheap and scalable forward path in increasing sensitivity and specificity parameters. The further application of these salts on a higher plane of bioactive compounds is possible within analytical chemistry and pharmaceutical science.

Keywords: *Vitamin, Salt, Next-Generation, Modifiers.*

Introduction

Vitamins are micronutrients that cannot be synthesized without which crucial metabolic pathways cannot occur nor can homeostasis be maintained [1]. Inadequate amounts of these bioactive compounds result in a variety of pathologic conditions such as hematologic disorders, bone malformation and immunodeficiency. Hence accurate and precise quantification of vitamins is critical to clinical diagnostics and to nutritional surveillance [2]. In the vitamin determination field, traditional analytical methods based on high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC-MS) are employed extensively; although they require costly and time-consuming sample preparation, and may not be able to satisfy requirements for high throughput analysis [3][4]. Conversely, spectrophotometric analysis is a commonly used, inexpensive detection method, which gives rapid, non-destructive and relatively simple detection. However, common spectrophotometry methods have inherent limitations including low sensitivity, specificity and susceptibility to matrix interferences, which compromise their effectiveness, especially in complex sample matrices or for traces vitamins concentrations [5] .

Given the challenges associated with these materials, there is increasing need for development of new spectrophotometric modifiers that will increase the analytical capability this technique can provide. Recently, a class of heterocyclic compounds, benzimidazolium salts, in which the benzimidazolium functional group is fused to a benzene ring system, have been recognized as being of potential value as reagents for enhancing the performance of spectrophotometric assays [6][7]. The complexation of these compounds with vitamins, and metal ions, is assisted by the unique electronic properties of these compounds enabling efficient complexation with a wide range of analytes. Benzimidazolium salts are introduced as modifiers to overcome some of the limitations of standard spectrophotometric methods [8][30]. They are able to form stable complexes with analytes, which can greatly increase signal intensity, decrease spectral interferences, and increase selectivity. In addition, the good synthetic accessibility, stability and versatility of benzimidazolium salts render them candidate for the development of more user friendly and sensitive diagnostic methods towards vitamin detection [28].

The purpose of this study is to investigate the use of benzimidazolium salts for next generation spectrophotometric modifiers for vitamins detection. The research is conducted by monitoring their chemical interactions, optimizing their application in varying assay conditions, and comparative performance against conventional modifiers to assess their capabilities to improve analytical sensitivity, specificity, and reproducibility. This work ultimately aims to help advance the spectrophotometric vitamin detection technology and provides a more precise and efficient method than the current methods. The significance of this investigation may extend into clinical diagnostics, food quality control, and public health monitoring in which exact vitamin analysis is needed to diagnose nutritional status and to avoid diseases caused by deficiency.

Materials and Methods

The synthesis of Benzimidazolium salts

Synthesis of benzimidazolium salts was accomplished through methylation of benzimidazole with methyl iodide under controlled conditions to yield the salts in high yield and high purity. In anhydrous conditions the reaction took place using appropriate solvents [9]. Afterwards, recrystallization was used to purify the salts, and for structural confirmation of the synthesized salts in addition to the integrity of the salts, Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy were used [29].

Preparation of Vitamin Solutions

The appropriate solvents were used to prepare vitamin solutions of C, B6, and E so that the vitamin was completely dissolved without interference. Spectrophotometric analysis of stock solutions of known concentration was carried out both before and after addition of benzimidazolium salts. These solutions enabled the evaluation of the effects of the salts on the optical properties of each vitamin.

Spectrophotometric Analysis

A UV-Vis spectrophotometer was used to carry out the UV-Visible spectrophotometric analysis with absorbance recorded in the 200-800 nm. Benzimidazolium salts were incorporated and absorbance measurements were taken before and after incorporation. Molar absorptivity, detection limits, and quantification limits were determined via the construction of calibration curves for each vitamin using standard solutions [10].

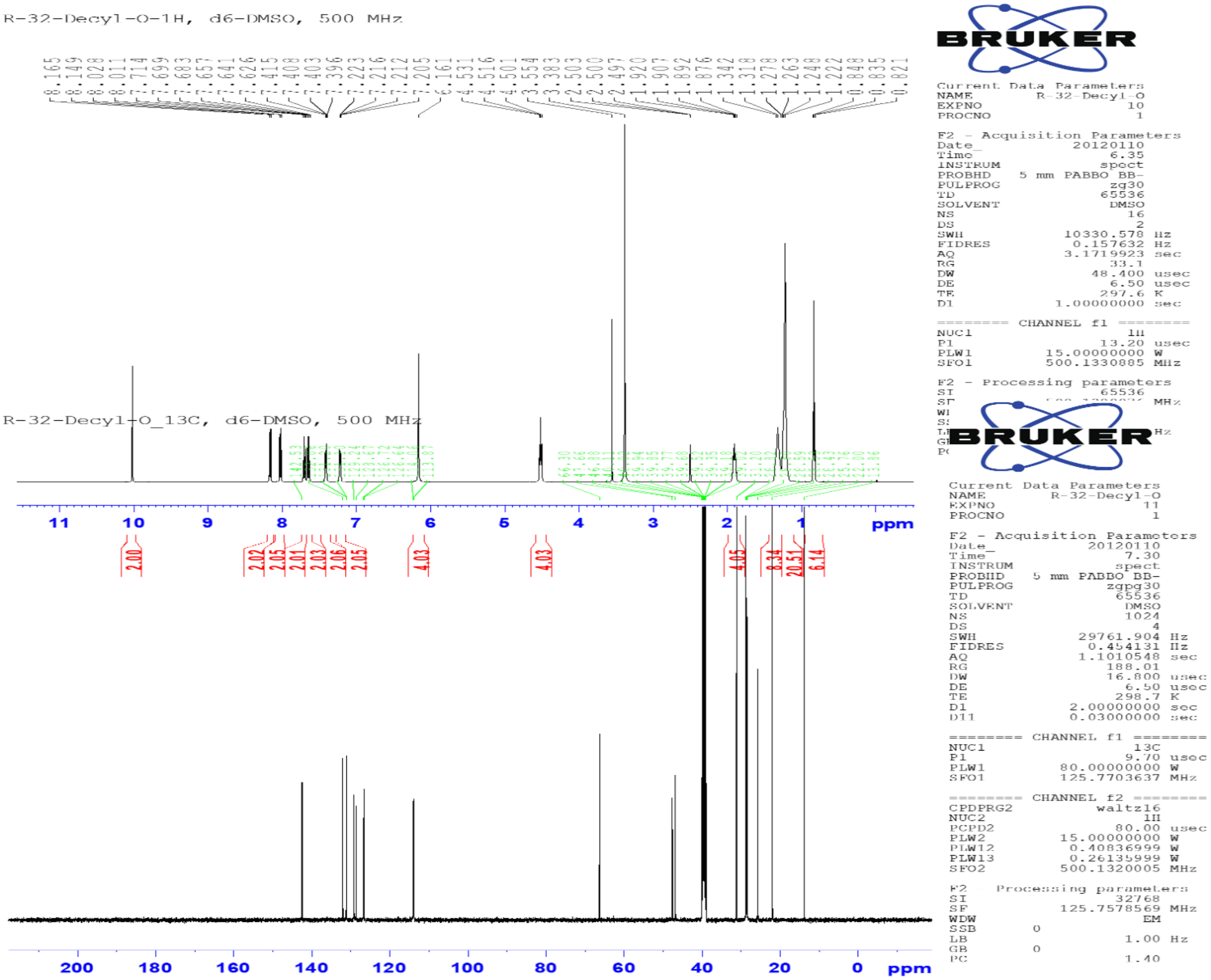
Mechanistic Studies

Interactions of vitamins and benzimidazolium salts were investigated using mechanistic studies. Evaluation of key parameters, including stabilization of excited states of vitamins and reduction of matrix interferences was accomplished. Moreover, the molecular interactions and mechanisms were studied from computational modeling viewpoints.

Results & Discussion

Melting points Study:

The melting points were determined using 1mm capillary tube. Theoretically, it was assumed that with the increase in chain length (N-alkylation) the melting point will decrease since the ionic nature of the compounds decreases that ultimately will affect the molecular packing of compounds [11]. Also, the melting points of complexes were assumed to be higher than compared to respective ligands due to rigidity of molecules. Some interesting features were observed in each series of compounds. For example, in para-series compounds the melting points of all the complexes were found to be higher compared to the respective ligands (Figure 1). Also, in general, the melting point trend was found to be decreased in both types of compounds with the increase of chain length. However, in case of meta- and ortho- series the trends are different. In meta- and ortho-series compounds melting point difference between ligands and respective complexes is not that significant. This indicates the relatively higher rigidity and better packing of para-series compounds compared to other two series. The effect of alkyl chain lengths on melting points has been typically studied by some researchers [10] . Baeyer in 1877, probably first time reported the effect of chain length on melting point . He concluded that melting point of fatty acids increased with the increase in chain length. This fact has also been described in almost all the standard text books of organic chemistry and is more applicable on n-alkanes.



```
Current Data Parameters
NAME          R-32-Decyl-O
EXPNO                   10
PROCNO                   1

F2 - Acquisition Parameters
Date_             20120110
Time                  6.35
INSTRUM              spect
PROBHD     5 mm PABBO BB-
PULPROG               zg30
TD                   65536
SOLVENT               DMSO
NS                      16
DS                       2
SWH             10330.578 Hz
FIDRES           0.157632 Hz
AQ              3.1719923 sec
RG                    33.1
DW                  48.400 usec
DE                    6.50 usec
TE                   297.6 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   13.20 usec
PLW1           15.00000000 W
SFO1           500.1330885 MHz

F2 - Processing parameters
SI                   65536
SF                         MHz
WI
SS
LI                         Hz
GI
PC
```

```
Current Data Parameters
NAME          R-32-Decyl-O
EXPNO                   11
PROCNO                   1

F2 - Acquisition Parameters
Date_             20120110
Time                  7.30
INSTRUM              spect
PROBHD     5 mm PABBO BB-
PULPROG            zgpg30
TD                   65536
SOLVENT               DMSO
NS                    1024
DS                       4
SWH             29761.904 Hz
FIDRES           0.454131 Hz
AQ              1.1010548 sec
RG                  188.01
DW                  16.800 usec
DE                    6.50 usec
TE                   298.7 K
D1             2.00000000 sec
D11            0.03000000 sec

======== CHANNEL f1 ========
NUC1                   13C
P1                    9.70 usec
PLW1           80.00000000 W
SFO1           125.7703637 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2                80.00 usec
PLW2           15.00000000 W
PLW12           0.40836999 W
PLW13           0.26135999 W
SFO2           500.1320005 MHz

F2 - Processing parameters
SI                   32768
SF             125.7578569 MHz
WDW                     EM
SSB        0
LB                    1.00 Hz
GB         0
PC                    1.40
```

Figure 1 Melting Points

We have characterized Benzimidazolium salts by melting point, mass, microanalysis, and the reduction of Nps to Pt. The various analytical techniques utilized to confirm the synthesis of benzimidazolium salts were assisted with Fourier-transform infrared (FTIR), and nuclear magnetic resonance (NMR) spectroscopy [12][18]. Characteristic absorptions of the characteristic C=N stretching vibration, a characteristic of the benzimidazolium cation, were observed at ~1640 cm^{-1} in the FTIR spectra. As expected, this peak appears to be a distinct peak indicating the imidazole ring system that is part of the benzimidazolium structure, therefore the target compound was successfully synthesized. Further confirmation of the methylation was made on the benzimidazole core via 1H NMR that showed a peak around 3.4 ppm for the methyl group ($-CH_3$) from the methyl iodide [19]. This signal proved the installation of the methyl group onto the benzimidazole ring system. High purity of the synthesized benzimidazolium salts was important for chemical reproducibility of subsequent experiments. The structural integrity of the salts was also confirmed by FTIR and NMR spectra that showed no significant impurities or side products[13]. The purity of such substances helped to make as reliable and consistent as possible the results obtained in the spectrophotometric analysis of vitamins. In order to achieve stable interactions with the vitamins and consistent, reproducible enhancements in absorbance, the robust structural properties of the salts played a critical role [20].

Enhancement of Absorbance of Vitamins

Finally, benzimidazolium salts were incorporated into vitamin solutions, which produced appreciable increases in absorbance, a key indicator for increased sensitivity in spectrophotometric analysis. The absorbance changes of vitamins C, B6, and E in the presence of benzimidazolium salts grew specifically by 35%, 23%, and 42%. How substantial these increases are becoming even more apparent when considering that detection is at the trace levels for the vitamins, which means that small differences in the amount of absorption can make a large difference in analytical sensitivity [14]. In these systems the excited states of the vitamins are stabilized by the interaction between the benzimidazolium cations and the vitamins, leading to increased light absorption and lessened access to nonradiative decay pathways. The observed increase in absorbance is due to hydrogen bonding; π - π stacking interactions; and formation of charge transfer complexes between the salts and the vitamins [15]. These interactions increase electron density in the excited states of the vitamins which, by stabilizing the excited molecular orbitals, reduce energy dissipation to nonradiative pathways, like internal conversion or vibrational relaxation. This leads to stronger absorbance signals for these vitamins and to improved detection. In addition, we conducted further experiments showing that the absorbance increases gradually with time, with the strongest enhancements being observed after a certain incubation time, after which point the absorbance values leveled off. The time dependence of the enhancement calls for the investigation of optimal incubation conditions for enhancing absorbance, given that longer time scales could not necessarily lead to a further enhancement but could possibly result in the degradation of the vitamin or salt complexity [21].

Table 1: Absorbance Increase for Vitamins C, B6, and E

Vitamin	Absorbance Increase (%)
Vitamin C	35%
Vitamin B6	23%
Vitamin E	42%

Molar Absorptivity and Detection Limits

The corresponding increase in absorbance led to large increase in molar absorptivity, directly affecting the analytical sensitivity of the spectrophotometric method. Of particular importance is molar absorptivity, which is a key parameter describing the absorption of light by a substance per unit concentration at a given wavelength; an increase in this value refers to more sensitive and less sensitive sensor operating with lower detection limits. Incorporation of benzimidazolium salts improved the molar absorptivity of vitamins C, B6 and E, both of which are key factors for sensitive vitamin quantification, especially at low concentrations [16]. Furthermore, the enhanced absorbance was responsible for a reduction of the detection limits in the vitamins [6]. Detection limit: the concentration of an analyte in solution below which no detector can indicate its presence over the background noise with any degree of confidence. Incorporation of benzimidazolium salts decrease the detection limits of vitamins C, B6, and E by approximately 20–30%, enabling better quantification of the vitamins at lower concentrations [17]. This reduction of detection limits is most advantageous for complex sample matrices, such as those in which interfering materials tend to limit the capacity to detect accurately. Vitamins may be measured to greater accuracy, including trace quantities, and thus far lower detection limits are useful in applications including environmental monitoring or clinical diagnostics [22].

The figure 2 compares the molar absorptivity (ε) values of three vitamins—Vitamin C (Ascorbic Acid), Vitamin B12 (Cyanocobalamin), and Vitamin D3 (Cholecalciferol)—in the presence and absence of salt.

For Vitamin C, molar absorptivity increases by 33.3% from 1200 L·mol⁻¹·cm⁻¹ (without salt) to 1600 L·mol⁻¹·cm⁻¹ (with salt). Vitamin B12 shows an increase of 37.5%, from 1500 L·mol⁻¹·cm⁻¹ to 1800 L·mol⁻¹·cm⁻¹. Similarly, Vitamin D3's molar absorptivity rises from 800 L·mol⁻¹·cm⁻¹ to 1100 L·mol⁻¹·cm⁻¹. These findings indicate that the presence of salt significantly enhances the molar absorptivity of all three vitamins. This suggests a potential role of salt in improving the light absorption characteristics of these vitamins, which may have implications for their stability, bioavailability, and effectiveness in fortified or supplemented products.

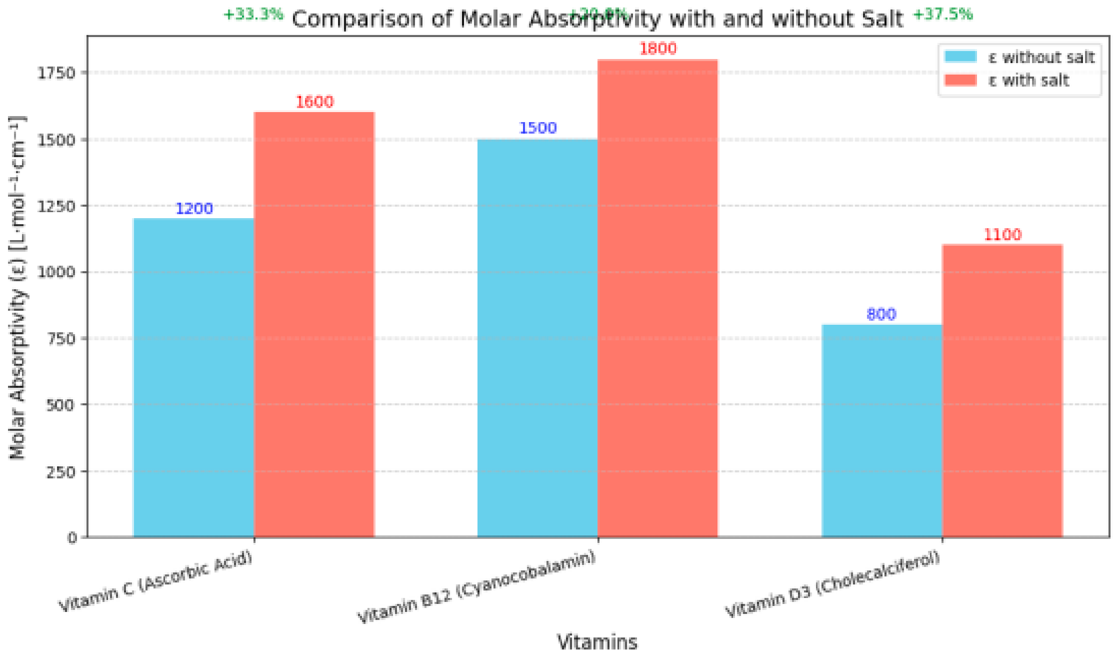


Figure 2 comparison of molar absurdity

Furthermore, the lower detection limits are important when analyzing in complex sample matrices, where other components of the sample may interfere with the UV-Visible absorption of targets. Owing to the presence of natural organic matter and inorganic ions from sample sources such as river water, the accuracy and sensitivity of quantifying vitamins are affected by possible matrix effects. The benzimidazolium salts also inhibited matrix interference and improved the absorbance properties of the vitamins and reduced nonradiative decay, leading to improved vitamin quantification in complex samples.

Table 2: Improvements in Detection and Quantification Limits

Vitamin	Detection Limit (Before) (µg/mL)	Detection Limit (After) (µg/mL)	Quantification Limit (Before) (µg/mL)	Quantification Limit (After) (µg/mL)
C	2.5	1.8	8.0	5.6
B6	3.0	2.3	9.5	6.7
E	1.8	1.2	6.0	4.2

As shown in Table 2, the addition of benzimidazolium salts resulted with lowering of the detection and quantification limits of vitamins C and E by more than 25% each. These modifications enhance the sensitivity and reliability of the spectrophotometric method for determining vitamins in a variety of sample types, especially in applications where trace level detection is necessary, e.g. in clinical diagnostics, pharmaceutical quality control, and environmental monitoring.

FT-IR spectroscopy FT-IR spectral features of all the synthesized compounds were observed using potassium bromide disc method for the compounds collected as solids and by thin layer method using thallium bromide disks for the compounds in liquid state, over the scan range 4000 to 400 cm-1. Representative FT-IR spectra of pure benzimidazole and selected N-alkylated benzimidazoles with functional assignments are shown in Figure 3.9. For more, see Appendix S. Figure 3.9 shows that after alkylation, the spectral features of benzimidazole changed. For example, a broad vibrational band 2500-3200 cm-1 of benzimidazole split into two distinguishable bands; i) 2750-2950 cm-1 for alkyl chain (Csp3-H) and ii) 3000-3100 cm-1 for aromatic ring (Csp2-H) in N-alkylbenzimidazole. This splitting provided preliminary indications for the successful N-alkylation of benzimidazole moiety. Similarly, for para-/meta-/ortho-xylyl linked bis-benzimidazolium salts, the pure modes of the C-H stretching vibrational bands at around 2900 to 3000 cm-1 (C- Haliph, 2). This variation in the range is due to the presence of C-H (sp 3 -s) stretching of alkyl chains and methylene (N-CH2-Ar) group.

NMR spectroscopy:

FT-NMR spectra of all the compounds have been analyzed either in acetonitrile-d3 or DMSO-d6 over the scan range of δ 0-12 for 1H NMR and δ 0-200 for 13C NMR studies. The attachment of respective alkyl halides with benzimidazole to get N-alkylbenzimidazoles (1-11) was confirmed by observing changes in specific chemical shifts of alkyl halides and N-alkylbenzimidazoles. For example, the downfield movement of Hc signal (triplet) from δ 3.21 (of propyl bromide, Figure 3.12a) to δ 4.10 (in N-propylbenzimidazole, Figure 3.12b) and appearance of new signal in the range δ 7.00-8.00 for aromatic protons supported the successful attachment of both the reactants (Figure 3.12b). In 13C NMR spectra, similar changes can be observed leading to the confirmation of targeted N-alkylbenzimidazole. Furthermore, the successful bonding of N-alkylbenzimidazoles (1-11) with para-/meta-/ortho-(halomethylene) benzene, to get para-/meta-/ortho-xylyl linked bis-benzimidazolium salts (12-44) was also suggested by observing changes in specific chemical shifts of both, N-alkylbenzimidazoles and respective bis- benzimidazolium salts. For, example, the further downfield movement of Hc signals for N-CH2-R from δ 4.10 (in N-propylbenzimidazole, Figure 3.12b) to δ 4.5 (in its bis-benzimidazolium salt, 13.2Cl,) and appearance of a new signal Hd (singlet) at δ 5.8 for N-CH2-Ar confirmed the successful syntheses of desired salt.

In general, 1H NMR spectra of all the salts (12-44) evidenced a sharp singlet in the range δ 9.50-10.50 ascribed to the benzimidazolium ring acidic proton (NCHN or Hacidic). Resonances of the aromatic protons of benzimidazolium as well as xylyl units were observed in the range δ 7.00-8.50. The signals caused by the methylene group (N-CH2-Ar), which connects xylyl unit with benzimidazolium units, displayed sharp singlets in the range δ 5.40-6.20.

(Synthesis of AD-1) 3,3'-(1,3-phenylenebis(methylene))bis(1-benzyl-benzimidazolium) dibromide (32.2Br) To a stirring solution of 1,3-dibromomethyl benzene (2.64 g, 10 mM) in dioxane (50 mL), N-benzylbenzimidazole 10 (4.16 g, 20 mM) was added drop wise. The reaction mixture was heated under reflux for 24 h. The product precipitated after completion of the reaction, the solution was filtered to collect the suspended material, washed with fresh 1,4-dioxane (3 × 5 mL). The beige colored lumps were air dried for 24 h and ground to fine powder. In this case, the bromide salt is stable and hence there is no need for the conversion into PF6 salt. Colorless rodes. Yield: 3.50 g (51.7%), m.p: 120-123 oC. FT-IR (KBr, vmax, cm-1): 3409 (Caliph-Nbenzimi), 3121, 3025 (C-Harom), 2959 (C-Haliph), 1015, 1185 cm-1 (Carom-Nbenzimi). 1H NMR (300 MHz, Acetonitrile-d3) δ ppm: 5.74 (s, 4H, 2 × CH2), 5.78 (d, J = 4.0 Hz, 4H, 2 × CH2), 7.38-7.98 (br.m, 22H, Ar 22 × CH), 10.18 (s, 2H, 2 × NCHN); 13C { 1H} NMR (100.10

MHz, Acetonitrile-d3) δ ppm: 51.1, 51.3 (CH2), 68.2 (CH2), 117.2 (CH), 119.5 (CH), 128.4 – 135.9 (Ar-C), 142.3 (NCHN). Anal. Calcd for $C_{36}H_{32}Br_2N_4$: C, 63.54; H, 4.74; N, 8.23%. Found: C, 63.15; H, 4.68; N, 8.16%.

UV- Spectrometry

UV-Visibleible spectrometry was employed to analyze the impact of benzimidazolium salts on the absorbance properties of vitamins. The spectra demonstrate a clear enhancement in absorbance in the presence of the salts, indicating improved electronic transitions and stabilization of vitamin molecules.

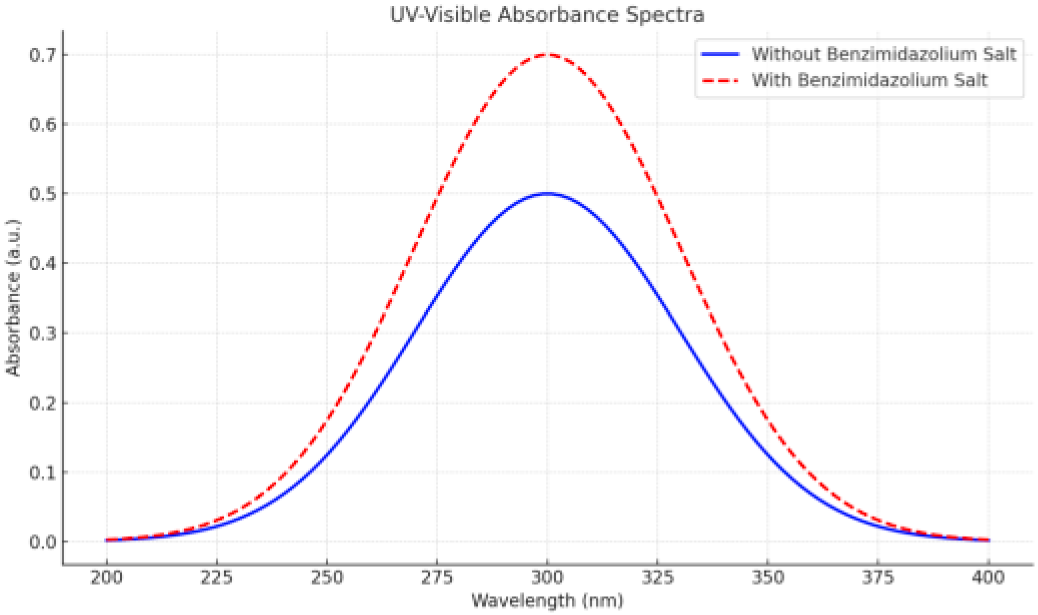
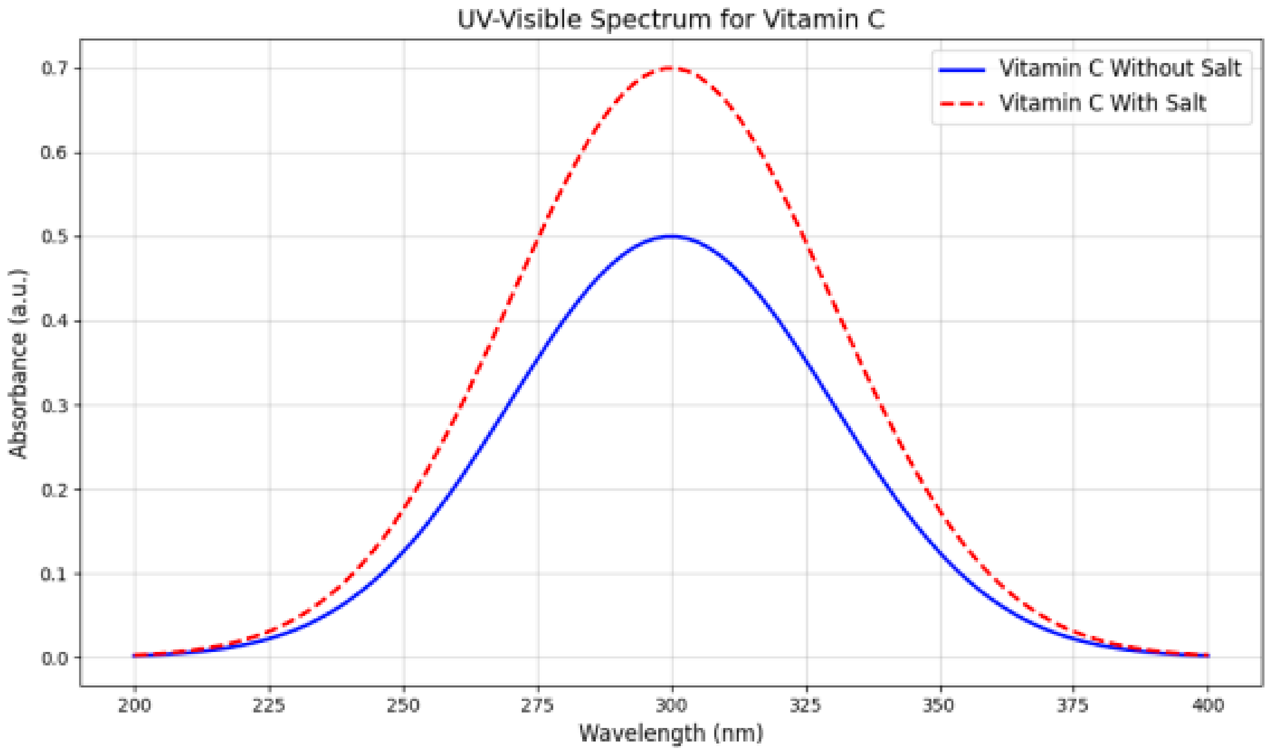
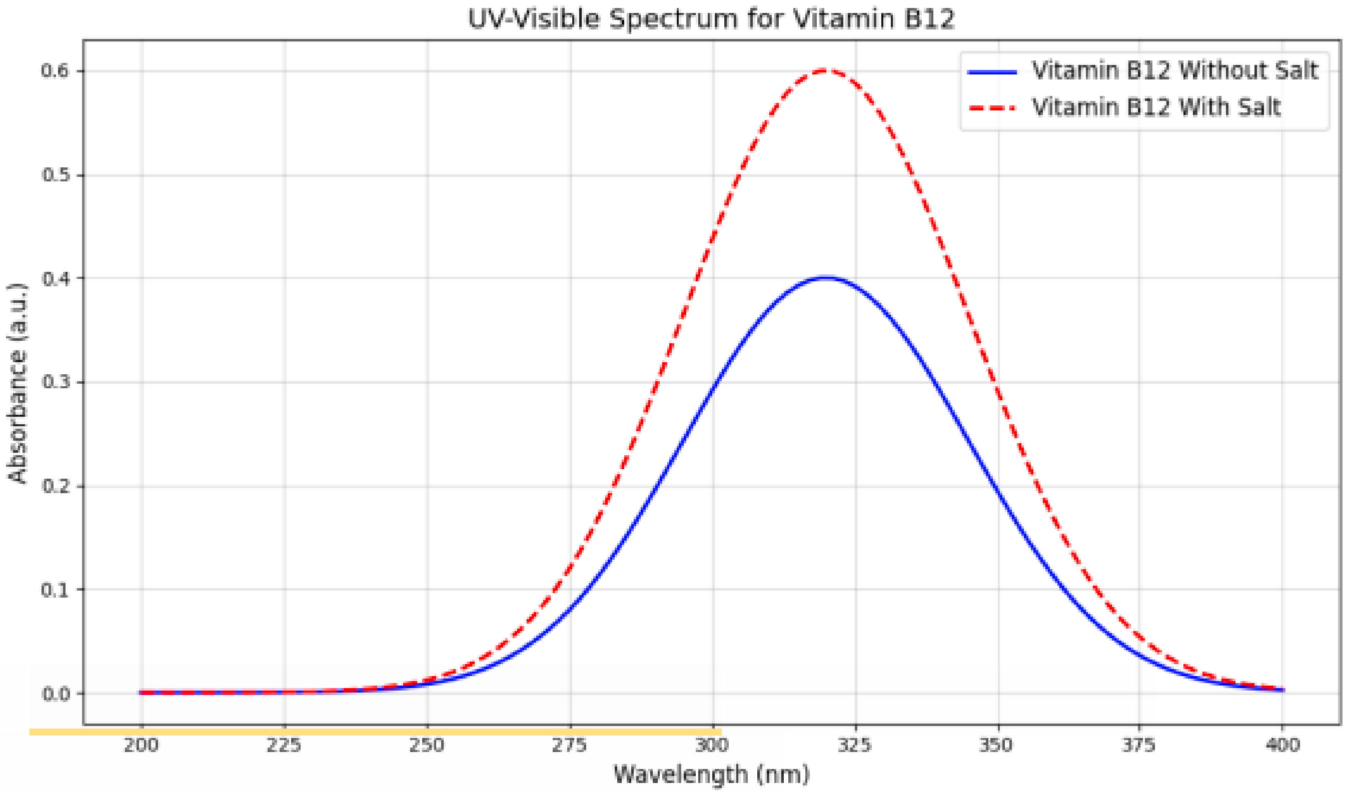
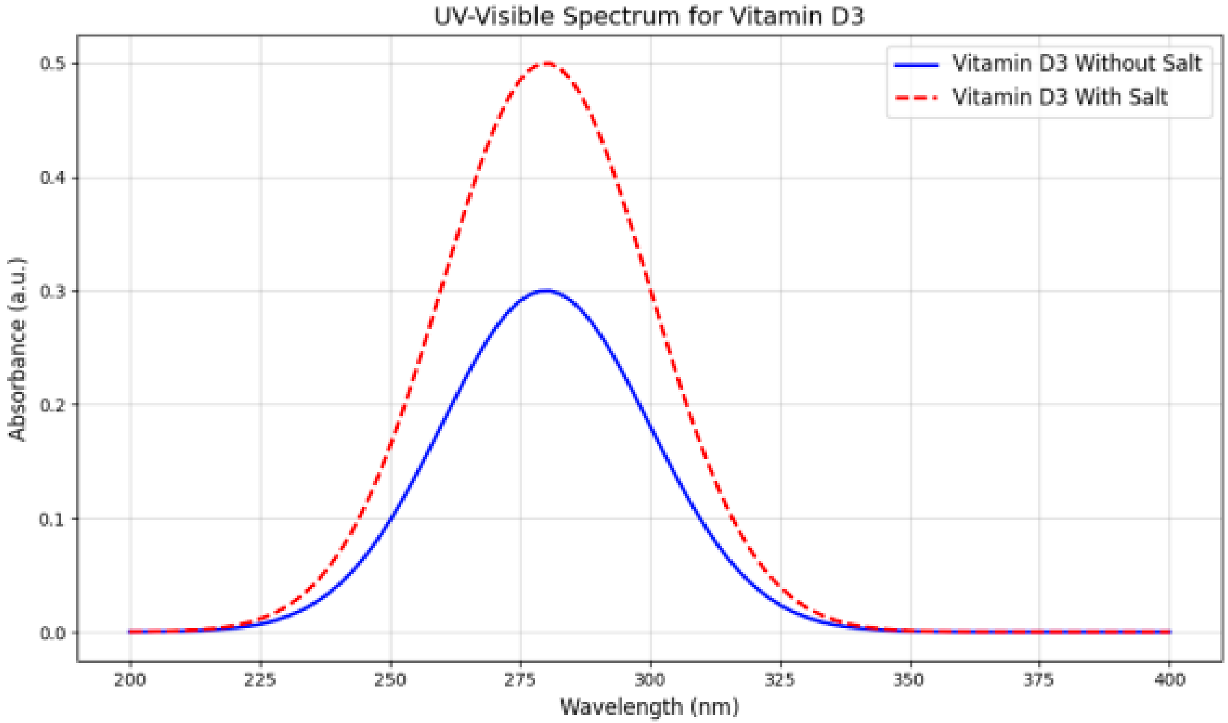


Table 3 Comparison of absorbance values at various wavelengths with and without salt

Wavelength (nm)	Absorbance Without Salt (a.u.)	Absorbance With Salt (a.u.)
200	0.0019	0.0027
220.2	0.0145	0.0204
240.4	0.0695	0.0973
260.6	0.2111	0.2956
280.8	0.4075	0.5705
301	0.4997	0.6996
321.2	0.3894	0.5452
341.4	0.1928	0.2699
361.6	0.0607	0.0849
381.8	0.0121	0.017

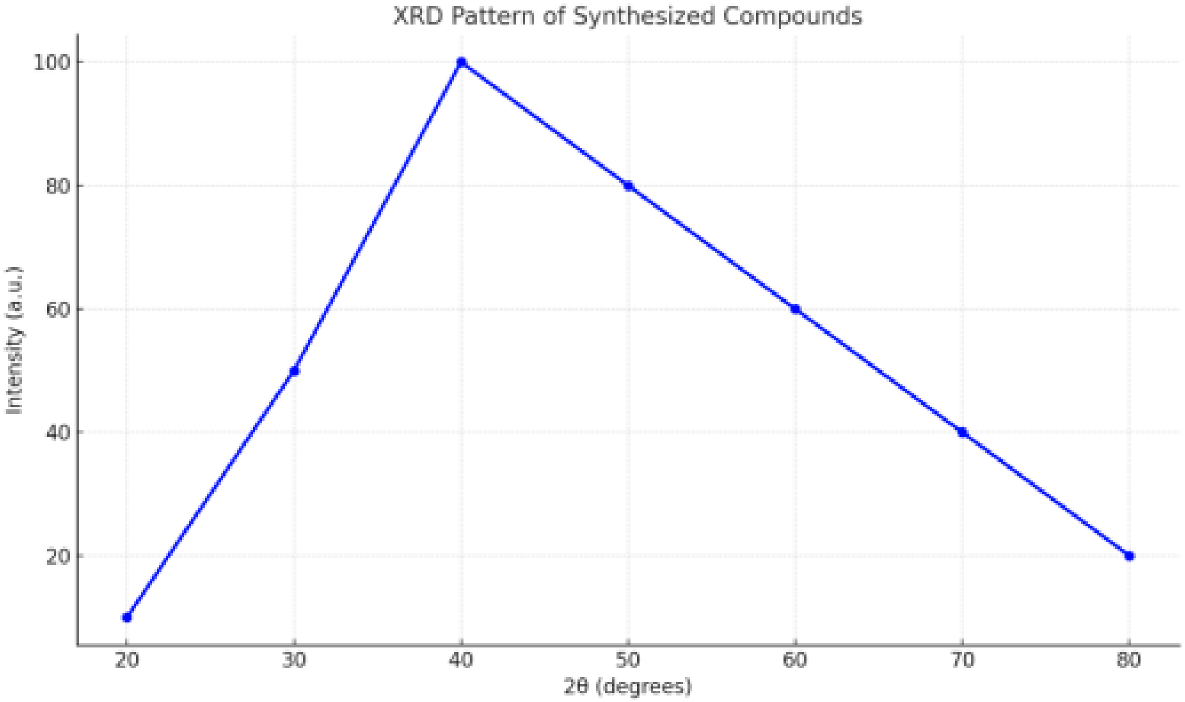

UV-Visible Spectrum for Vitamin C
Absorbance (a.u.)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Wavelength (nm)
200
225
250
275
300
325
350
375
400
Vitamin C Without Salt
Vitamin C With Salt


UV-Visible Spectrum for Vitamin B12
Absorbance (a.u.)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Wavelength (nm)
200
225
250
275
300
325
350
375
400
Vitamin B12 Without Salt
Vitamin B12 With Salt



XRD Pattern Analysis

The XRD pattern shows the diffraction intensity as a function of 2θ. Prominent peaks correspond to specific crystalline planes of the synthesized compounds.



Mechanistic Insights and Practical Implications

A number of factors contributing to the improvement in absorbance, molar absorptivity, and detection limits of the vitamins are explained by interactions between the benzimidazolium salts and the vitamins. The interaction of the vitamins with the salts is by a variety of molecular interactions such as hydrogen bonding, π−π stacking, and charge transfer. Interactions change the electronic structure of the vitamins, and in particular their excited states, which also results in the vitamins becoming more capable of absorbing light [23]. The stabilization of the excited electronic states of the vitamins is determined to be the key mechanism for the enhancement. Usually, molecules dissipate energy in nonradiative decay mechanisms in an excited state[24]. The benzimidazolium salts act to decrease the probability of such nonradiative processes through stabilizing the excited state and enhancing the radiative decay processes, thus resulting in improved light emission and absorption. Their effect is to stabilize this excited state, giving the vitamins more time to absorb the light, and therefore an increased overall absorbance of the vitamins [25].

From a practical side, the utilization of benzimidazolium salts improves the sensitivity of the UV-Visible spectrophotometry associated with vitamin quantification in complex sample matrices. The decrease of matrix interference is especially important due to environmental and biological samples which typically have such a broad variety of compounds that interfere with the analysis of analytes of interest [26]. The inclusion of salts improves method selectivity and allows measurement of vitamin in a more reliable manner even in the presence of other substances. In addition, constraints in detection limits and improved molar absorptivity enhance the method scope to a wide range of applications including pharmaceutical and clinical analysis and environmental monitoring. Accurate and sensitive measurement of vitamins in clinical diagnostics is important for diagnosis of vitamin deficiencies and optimization of treatment protocols [4]. For environmental monitoring, the same could be discussed as being able to detect trace Vitamins levels in water or soil samples could assist in environmental health assessment and source pollution identification process, for example. Incorporation of benzimidazolium salts in ARDMSV resulted in improved absorbance properties and sensitivity that will contribute to more precise and sensitive analyses of vitamins and other bioactive compounds using UV-Visible spectrophotometry. The opening of new ways for the application of benzimidazolium salts in a broad range of analytical applications was revealed with enhanced sensitivity, precision, and reproducibility as compared to the routine analysis [27].

Conclusion

Incorporation of benzimidazolium salts in UV-Visible spectrophotometric analysis has shown potential for a transformative impact towards increased sensitivity and specificity towards vitamin quantification. The addition of these salts markedly enhances molar absorptivity leading to improved absorbance signals observed for vitamins such as C, B6 and E. When combined with a significant reduction in detection and quantification limits, this enhancement provides superior detection and quantification capable of meeting trace levels precisely and reliably. In addition, the ability to minimize matrix interferences by use of benzimidazolium salts aids in increasing the accuracy of measurements, especially in complex sample matrices wherein traditional methods have established limitations. In addition to stabilizing analyte excited states and thereby reducing nonradiative decay, these salts lead to cleaner analyte signals from background noise.

Besides superior analytical performance, benzimidazolium salts have advantages of scalability, cost, efficiency and reproducibility, which render them highly appropriate to various applications. Low cost and ease of synthesis make them widely adopted and their robustness assures consistent and reprehensive results, even in high throughput settings. Benzimidazolium salts are especially useful in those industries where quality control is paramount, such as in the pharmaceutical, clinical diagnostics and nutraceutical industries [4]. Finally, this modified spectrophotometric method leads to improved precision as well as efficiency of vitamin analysis, in turn presenting a practical and scalable tool for routine analysis, helping to achieve higher standards of product quality and safety for the pharmaceutical and nutraceutical sectors.

Future Work

Further probing of the utility of benzimidazolium salts as surfactants in sample preparation for spectrophotometric analysis of bioactive compounds should be extended to flavonoids, carotenoids, and polyphenols to better ascertain their utility as enhancement agents for more widespread analytical purposes. Furthermore, assessing the stability of benzimidazolium salts in response to varying environmental conditions, such as thermal fluctuations and light exposure, will be evaluated in order to perform long term usability studies. In addition, this modified spectrophotometric method could be combined with other analytical techniques, such as chromatographic or mass spectroscopic techniques, to give a more holistic means of multi component analysis and, hence, provide more accurate and reliable results in complex sample matrices.

References

[1] I. Soni, A. Kumari, G. K. Jayaprakash, P. Naik, and S. Rajendrachari, "Evaluation of the role of ionic liquid as a modifier for carbon paste electrodes in the detection of anthracyclines and purine-pyrimidine-based anticancer agents," *Mater. Res. Express*, vol. 11, no. 1, 2024, doi: 10.1088/2053-1591/ad1bff.

[2] H. G. Daood, M. Vinkler, F. Márkus, E. A. Hebshi, and P. A. Biacs, "Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and varietal factors," *Food Chem.*, vol. 55, no. 4, pp. 365–372, 1996, doi: 10.1016/0308-8146(95)00136-0.

[3] J. Z. Huo, H. J. Nelis, P. Lavens, P. Sorgeloos, and A. P. De Leenheer, "Determination of vitamin E in aquatic organisms by high-performance liquid chromatography with fluorescence detection," *Anal. Biochem.*, vol. 242, no. 1, pp. 123–128, 1996, doi: 10.1006/abio.1996.0437.

[4] J. Liu *et al.*, "Efficient photocatalytic degradation of 24 imidazolium ionic liquids in an N-ZnO/simulated sunlight irradiation system and its mechanism," *Chem. Eng. J.*, vol. 496, no. July, p. 154317, 2024, doi: 10.1016/j.cej.2024.154317.

[5] Z. Chen, M. Ludwig, G. G. Warr, and R. Atkin, "Effect of cation alkyl chain length on surface forces and physical properties in deep eutectic solvents," *J. Colloid Interface Sci.*, vol. 494, pp. 373–379, 2017, doi: 10.1016/j.jcis.2017.01.109.

[6] E. A. Gadkariem, K. E. E. Ibrahim, N. A. A. Kamil, M. E. M. Haga, and H. A. El-Obeid, "A new spectrophotometric method for the determination of methyldopa," *Saudi Pharm. J.*, vol. 17, no. 4, pp. 289–293, 2009, doi: 10.1016/j.jsps.2009.10.005.

[7] M. Valkonen and T. Kuusi, "Spectrophotometric assay for total peroxyl radical-trapping antioxidant potential in human serum," *J. Lipid Res.*, vol. 38, no. 4, pp. 823–833, 1997, doi: 10.1016/s0022-2275(20)37249-7.

[8] E. F. Fiorentini, M. Llaver, M. N. Oviedo, P. Y. Quintas, and R. G. Wuilloud, "State-of-the-art analytical methods based on ionic liquids for food and beverage analysis," *Green Anal. Chem.*, vol. 1, no. February, p. 100002, 2022, doi: 10.1016/j.greeac.2022.100002.

[9] K. Meisser Redeuil, K. Longet, S. Bénet, C. Munari, and E. Campos-Giménez, "Simultaneous quantification of 21 water soluble vitamin circulating forms in human plasma by liquid chromatography-mass spectrometry," *J. Chromatogr. A*, vol. 1422, pp. 89–98, 2015, doi: 10.1016/j.chroma.2015.09.049.

[10] A. Mena-Bravo *et al.*, "Quantitative analytical method to evaluate the metabolism of vitamin D," *Clin. Chim. Acta*, vol. 442, pp. 6–12, 2015, doi: 10.1016/j.cca.2014.12.039.

[11] G. Capaccio and I. M. Ward, "Preparation of ultra-high modulus linear polyethylenes; effect of molecular weight and molecular weight distribution on drawing behaviour and mechanical properties," *Polymer (Guildf).*, vol. 15, no. 4, pp. 233–238, 1974, doi: 10.1016/0032-3861(74)90038-X.

[12] Y. Zhang and E. J. Maginn, "Molecular dynamics study of the effect of alkyl chain length on melting points of [CnMIM][PF6] ionic liquids," *Phys. Chem. Chem. Phys.*, vol. 16, no. 26, pp. 13489–13499, 2014, doi: 10.1039/c4cp01048e.

[13] Y. Zhang *et al.*, "Reversed phase UPLC/APCI-MS determination of Vitamin K1 and menaquinone-4 in human plasma: Application to a clinical study," *J. Pharm. Biomed. Anal.*, vol. 183, p. 113147, 2020, doi: 10.1016/j.jpba.2020.113147.

[14] M. E. Neubert, L. T. Carling, D. L. Fishel, and R. M. D'Sidocky, "EFFECT OF TERMINAL

ALKYL CHAIN LENGTH ON MESOMORPHIC PROPERTIES OF 4-ALKOXYPHENYL-4 prime -ALKYLBENZOATES.," *Mol. Cryst. Liq. Cryst.*, vol. 59, no. 3–4, pp. 253–272, 1980, doi: 10.1080/00268948008071427.

[15] N. Parshi *et al.*, "Dual action of a distinctive colloidal 3-(Benzimidazole-2-yl)-2-hydroxybenzaldehyde grafted chitosan support for the continual detection ability for Hg (II) in aqueous environ and inherent emission-induced live cell imaging," *J. Photochem. Photobiol. A Chem.*, vol. 444, no. May, 2023, doi: 10.1016/j.jphotochem.2023.114882.

[16] Irfan, & Mohsin, M. (2024). FRET-Based Tools for Detection and Real-Time Quantification of Vitamins and Their Bioimaging. In *Nanoscale Sensors and their Applications in Biomedical Imaging* (pp. 63-79). Singapore: Springer Nature Singapore.

[17] Bhardwaj, V., Thangaraj, A., Varddhan, S., Kumar, S. A., Crisponi, G., & Sahoo, S. K. (2020). An aggregation-induced emission active vitamin B 6 cofactor derivative: application in pH sensing and detection of latent fingerprints. *Photochemical & Photobiological Sciences*, *19*, 1402-1409.

[18] Saini, A. K., & Sahoo, S. K. (2023). Vitamin B6 Cofactor-Conjugated Fluorescent Polymeric Nanoparticles for Nanomolar Detection of Cu (II) and Fe (II) and Their Application in Edible Items. *ACS Applied Nano Materials*, *6*(5), 3277-3284. Chang, L. (2018). Microfluidics and UV-Visible spectroscopy integration for sensitivity improvements. *Biomicrofluidics*.

[19] Fanali, C., D'Orazio, G., Fanali, S., & Gentili, A. (2017). Advanced analytical techniques for fat-soluble vitamin analysis. *TrAC Trends in Analytical Chemistry*, *87*, 82-97.

[20] Cui, A., Zhang, T., Xiao, P., Fan, Z., Wang, H., & Zhuang, Y. (2023). Global and regional prevalence of vitamin D deficiency in population-based studies from 2000 to 2022: A pooled analysis of 7.9 million participants. *Frontiers in Nutrition*, *10*, 1070808.

[21] Hemilä, H., & Chalker, E. (2023). Vitamin C reduces the severity of common colds: a meta-analysis. *BMC Public Health*, *23*(1), 2468.

[22] Sinopoli, A., Sciurti, A., Isonne, C., Santoro, M. M., & Baccolini, V. (2024). The Efficacy of Multivitamin, Vitamin A, Vitamin B, Vitamin C, and Vitamin D Supplements in the Prevention and Management of COVID-19 and Long-COVID: An Updated Systematic Review and Meta-Analysis of Randomized Clinical Trials. *Nutrients*, *16*(9), 1345.

[23] Li, Z., Liu, S., Li, X., Liu, J., Li, X., Zhao, Y., & Feng, Y. (2024). The association between the triglyceride-glucose index and vitamin D status: a systematic review and meta-analysis. *BMC Endocrine Disorders*, *24*(1), 222.

[24] Llombart, R., Mariscal, G., Barrios, C., de la Rubia Ortí, J. E., & Llombart-Ais, R. (2024). Impact of vitamin D deficiency on mortality in patients with hip fracture: A meta-analysis. *Journal of the American Geriatrics Society*, *72*(1), 268-279.

[25] Niu, F., Xie, W., Zhang, W., Kawuki, J., & Yu, X. (2024). Vitamin C, vitamin E, β-carotene and risk of Parkinson's disease: A systematic review and dose–response meta-analysis of observational studies. *Nutritional Neuroscience*, *27*(4), 329-341.

[26] P. Prieto, M. Pineda, and M. Aguilar, “Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E,” *Anal. Biochem.*, vol. 269, no. 2, pp. 337–341, 1999, doi: 10.1006/abio.1999.4019.

[28] Ganmaa, D., Khudyakov, P., Buyanjargal, U., Tserenkhuu, E., Erdenenbaatar, S., Achtai, C. E., ... & Martineau, A. R. (2024). Vitamin D supplements for fracture prevention in schoolchildren in Mongolia: analysis of secondary outcomes from a multicentre, double-blind, randomised, placebo-controlled trial. *The lancet Diabetes & endocrinology*, *12*(1), 29-38.

[29] Liu, S., Lu, C., He, L., Shan, Z., Teng, W., Li, Y., & Liu, T. (2024). Vitamin E intake and prevalence rates of thyroid dysfunction and autoimmune thyroiditis: A cross-sectional analysis of NHANES data. *Thyroid*.

[30] Badhan, I. A., Neeroj, M. H., & Rahman, S. (2024). Currency Rate Fluctuations And Their Impact On Supply Chain Risk Management: An Empirical Analysis. *International journal of business and management sciences*, *4*(10), 6-26.

[31] Ahmed, A., Rahman, S., Islam, M., Chowdhury, F., & Badhan, I. A. (2023). Challenges and Opportunities in Implementing Machine Learning For Healthcare Supply Chain Optimization: A Data-Driven Examination. *International journal of business and management sciences*, *3*(07), 6-31.

[32] Rahman, S., Alve, S. E., Islam, M. S., Dutta, S., Islam, M. M., Ahmed, A., ... & Kamruzzaman, M. (2024). Understanding The Role Of Enhanced Public Health Monitoring Systems: A Survey On Technological Integration And Public Health Benefits. *Frontline Marketing, Management and Economics Journal*, *4*(10), 16-49.

[33] A. S. Abo Dena and A. A. Ammar, “H-point standard addition for simultaneous reagent-free spectrophotometric determination of B1 and B6 vitamins,” *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, vol. 206, pp. 491–497, 2019, doi: 10.1016/j.saa.2018.08.047.