IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 14, Issue 10 Series. I (October 2024), PP. 16-33
www.iosrphr.org

Inclusion Complexation of Vitamin B12 with Cyclodextrins for Light Stability

Ayesha Noorain K M *[1], Venkatesh [2], Parthasarathi K Kulkarani[3], Hanumanthachar Joshi K [4]

[1] *(Department Of Pharmaceutics, Saradavilas College Of Pharmacy, Mysuru,Karnataka,India).*
[2] *(Hod Of Pharmaceutics Sadavilas Collge Of Pharmacy, Mysuru,Karnataka,India).*
[3] *(Research Deen, Sadavilas Collge Of Pharmacy, Mysuru,Karnataka,India)*
[4] *(Principal, Sadavilas Collge Of Pharmacy, Mysuru,Karnataka,India)*
Received 26 October 2024; Accepted 13 November 2024

Abstract:
Vitamin B12, an essential micronutrient with multifaceted roles in human physiology, encounters significant challenges concerning its stability when exposed to light. The photolabile nature of Vitamin B12 poses a substantial concern in various industries, necessitating innovative strategies to safeguard its stability and efficacy. Cyclodextrins, notably beta-cyclodextrin and its derivative, hydroxypropyl beta-cyclodextrin, present promising capabilities as host molecules, forming inclusion complexes with guest compounds. This study investigates the potential of beta-cyclodextrin and hydroxypropyl beta-cyclodextrin to enhance the light stability of Vitamin B12 through inclusion complexation. The research aims to elucidate and compare the interactions between Vitamin B12 and these cyclodextrins, specifically focusing on their efficacy in shielding Vitamin B12 from photodegradation. Experimental methodologies include spectroscopic analyses, encapsulation efficiency studies, and stability assessments under controlled light conditions. This study focused on formulating stabilized vitamin B12 complexes using beta-cyclodextrin (β-CD) and hydroxypropyl-beta-cyclodextrin (HP-β-CD) in a 1:1 ratio. Compatibility assessments via FT-IR indicated minimal interaction with β-CD and stability with HP-β-CD. Differential Scanning Calorimetry (DSC) showed improved thermal stability of the complexes. Release profiles revealed that β-CD significantly enhanced vitamin B12 release, achieving over 100% within five minutes, while stability studies confirmed consistent drug content under light exposure. The outcomes seek to discern the optimal host-guest relationship, unraveling mechanisms underlying the formation of inclusion complexes and their role in preserving Vitamin B12 integrity. The significance of this research lies in its potential to offer insights into novel strategies for improving the light stability of Vitamin B12-containing formulations**.**
Key Word: Vitamin B12, Cyclodextrins, Inclusion Complexation, Light Stability, Photodegradation, Hydroxypropyl Beta-Cyclodextrin.

I. Introduction

Vita, meaning "life" in Latin, refers to vitamins, essential micronutrients crucial for various biological processes. They help maintain optimal health, support metabolism, and can prevent chronic diseases by acting as catalysts in energy production from lipids, proteins, and carbohydrates. Vitamins are categorized into two groups based on solubility: water-soluble (B and C) and fat-soluble (A, D, E, K). Vitamin B12, a vital micronutrient, is prone to degradation when exposed to light, posing challenges in pharmaceuticals and food fortification.

Cyanocobalamin, a synthetic form of vitamin B12, is used to treat deficiencies and plays critical roles in methylation reactions necessary for cell division and growth. This research focuses on the indications, mechanisms of action, pharmacokinetics, and safety profile of cyanocobalamin while addressing its importance in treating vitamin B12 deficiency and related disorders.

The pharmacokinetics of cyanocobalamin includes rapid absorption after injection, transport via specific binding proteins, and excretion primarily through the kidneys. Special considerations are necessary for patients with hepatic or renal impairment, as well as during pregnancy and breastfeeding. Understanding these factors is essential for optimizing vitamin B12 therapy in clinical practice.

Cyclodextrins (CDs) are water-soluble, non-toxic cyclic oligosaccharides composed of D-glucopyranoside units linked by α-1,4-glycosidic bonds. The most prevalent types are α-CD, β-CD, and γ-CD, containing 6, 7, and 8 glucose subunits, respectively. Their unique structure features a hydrophilic exterior and a hydrophobic cavity, enabling the formation of inclusion complexes with hydrophobic guest molecules. CDs have

diverse applications in bio detection, biomedicine, bioimaging, and agriculture. Recent advancements have focused on enhancing cyclodextrin performance and expanding their applications.

Notably, β-cyclodextrin and hydroxypropyl β-cyclodextrin have shown promise in stabilizing Vitamin B12 (cyanocobalamin) against light-induced degradation. Cyanocobalamin is particularly vulnerable to photodegradation when exposed to ultraviolet (UV) light, which can lead to a loss of potency and effectiveness due to the breakdown of its chemical structure. The photodegradation of cyanocobalamin involves mechanisms such as cobalt-carbon bond breaking and the formation of free radicals, which can significantly diminish its biological activity. This degradation not only reduces the vitamin's efficacy but also shortens the shelf life of formulations containing it.

Encapsulation of cyanocobalamin within cyclodextrins offers a protective strategy to mitigate these effects by limiting light exposure and stabilizing the molecular environment. This research aims to investigate the efficacy of β-cyclodextrin and hydroxypropyl β-cyclodextrin in forming inclusion complexes with Vitamin B12 to enhance its light stability. By exploring their encapsulation capabilities and molecular interactions, this study seeks to optimize the host-guest relationship for preserving Vitamin B12 integrity under light exposure. The findings from this investigation could provide valuable insights into utilizing cyclodextrins as effective carriers for Vitamin B12, potentially leading to more stable and efficacious formulations in pharmaceuticals and nutraceuticals.

II. Material And Methods

METHODOLOGY

- List of materials and equipment's.
- Pre-formulation studies.
- Formulation of stabilized Vitamin B12 powder
- Characterization & Evaluation of stabilized Vitamin B12.
- In vitro dissolution studies.
- Stability studies.

Pre-formulation Studies on Vitamin B12 and Cyclodextrin Complexes

1. **Organoleptic Properties:**

The color and odor of Vitamin B12 were characterized using descriptive techniques.

2. **Preliminary Solubility Analysis:**

Vitamin B12 solubility was tested in solvents like ethanol, methanol, chloroform, isopropyl alcohol, ethyl ether, and water. 1 mg of Vitamin B12 was dissolved in 10 mL of each solvent, stirred for 10 minutes using a magnetic stirrer.

3. **Determination Of Λ Max:**

Preparation of Vitamin B12 Solutions for λ Max Determination:

A stock solution of Vitamin B12 (10 mg/L) was prepared by dissolving 100 mg in 100 mL of water. Working solutions were then diluted from this stock to achieve concentrations from 1 to 7 µg/mL.

The UV spectrophotometric λ Max of Vitamin B12 was determined by scanning a standard solution (10 µg/mL) between 200-400 nm. The maximum absorption was recorded at 360 nm.

METHODS:

These Methods Are for Vitamin B 12 And Beta Cyclodextrins Complexation.

Vitamin B12 = 1355.4 grams per mole (g/mol).

Beta-Cyclodextrin =1134.987 grams per mole (g/mol).

I**. Kneading Method:**

Weigh Vitamin B12 and β-cyclodextrin in a 1:1 molar ratio.

Prepare a paste of β-cyclodextrin using a methanol (1:1) mixture.

Add Vitamin B12 and knead for 3 hours.

Dry the mixture at 45-50°C for 24 hours, then powder and sieve.

II. **Solvent Evaporation:**

Weigh Vitamin B12 and β-cyclodextrin (1:1 molar ratio).

Dissolve each in ethanol and mix.

Stir for 1 hour, evaporate solvent using a rotary evaporator, then dry the complex.

III. **Spray Drying:**

Dissolve Vitamin B12 and β-cyclodextrin (1:1 ratio) in water/ethanol.

Mix and spray dry at 150-200°C.

Collect the resulting powder.

IV. **Freeze Drying:**
Dissolve Vitamin B12 and β-cyclodextrin (1:1 ratio) in water.
Mix, freeze at -40°C, and freeze-dry.
Collect the dry powder.

These Methods Are for Vitamin B 12 And HP-Beta Cyclodextrins Complexation.
Vitamin B12 = 1355.4 grams per mole (g/mol).
HP-Beta-Cyclodextrin = 1375.4 grams per mole (g/mol).

I. **Kneading Method**:
Weigh Vitamin B12 and HP-β-CD in a 1:1 molar ratio.
Prepare a paste of HP-β-CD using ethanol or water.
Add Vitamin B12 and knead for 1-3 hours.
Dry the mixture at 40°C, then powder and store.
II. **Solvent Evaporation:**
Weigh Vitamin B12 and HP-β-CD (1:1 molar ratio).
Dissolve each in ethanol, mix, and stir for 1-2 hours.
Evaporate the solvent, then dry the solid complex at 40°C.
III. **Spray Drying:**
Dissolve Vitamin B12 and HP-β-CD in water/ethanol (1:1 ratio).
Mix thoroughly and spray dry at 150-200°C.
Collect the resulting powder.
IV. **Freeze Drying:**
Dissolve Vitamin B12 and HP-β-CD (1:1 ratio) in water.
Mix, freeze at -40°C, and freeze-dry.
Collect the dry powder.

COMPATIBILITY STUDIES:
A. FT-IR (Fourier Transform Infrared Spectroscopy):
FTIR analysis confirmed the identity and interaction between Vitamin B12 and cyclodextrin by comparing the spectra of pure and complexed forms.
B. Differential Scanning Calorimetry (DSC):
DSC was used to study the thermal properties of Vitamin B12 and its inclusion complexes, measuring melting points over a temperature range of -20°C to 180°C.

MICROMERITIC STUDIES:
A. Angle of Repose:
The flow property of the powder was measured using the fixed funnel method and calculated based on the pile's height and radius.
B. Bulk and Tapped Densities:
Bulk density was determined by measuring the volume occupied by the powder, while tapped density involved mechanically compacting the powder and measuring the resulting volume.
C. Hausner's Ratio:
This ratio was calculated to assess powder flowability, using tapped and bulk densities. Lower values indicate better flow properties.
IN-VITRO DRUG RELEASE STUDY
The *in-vitro* dissolution studies were performed using a USP Type-II dissolution apparatus. The Vitamin B12 inclusive complex with different cyclodextrins was placed in a tea bag and submerged in 900 ml of distilled water, maintained at 37 ± 0.5°C with a stirring speed of 50 rpm. Samples of 5 ml were collected at predetermined intervals, and the same volume of fresh medium was replaced. Sampling was done at 1, 2, 3, 4, and 5 minutes. The drug concentration released was estimated using a UV spectrophotometer at a wavelength of 360 nm.

STABILITY STUDIES
Stability studies of the optimized batch were conducted to assess changes in parameters like physical appearance, drug content, and in-vitro release profile during storage. The sample was exposed to short-term storage under D65 conditions (ISO 18909 standard for outdoor daylight) for 30 days. After 1.2 million lux hours, samples were withdrawn and evaluated for any changes in physical appearance and drug content.

III. Result

Pre-Formulation Studies Of Vitamin B12

Table 1. Organoleptic characteristics & Solubility of Vitamin B12.

PROPERTIES	REPORTED		OBSERVED
Appearance	Dark red crystals or an amorphous or crystalline red powder		an amorphous red powder
Odour	Odorless		Odorless
Solubility	Ethanol	Soluble	Soluble
	Methanol	Soluble	Soluble
	water	Soluble	Soluble
	DMSO	Soluble	Soluble
	acetone	Insoluble	Insoluble
	ether	Insoluble	Insoluble
	chloroform	Insoluble	Insoluble

Determination of λ max.

Solution of Vitamin B 12 (Cyanocobalamin) (100 µg/ml) was prepared using water and this solution was scanned for absorbance 200-800 nm using UV spectrophotometer (Shimadzu, UV-1900i Japan, UV-spectrophotometer). As shown in fig.1 peak was obtained at 360nm. The absorption maximum (λmax) was found 360nm. This value was selected for rest of the UV spectrophotometric analysis.

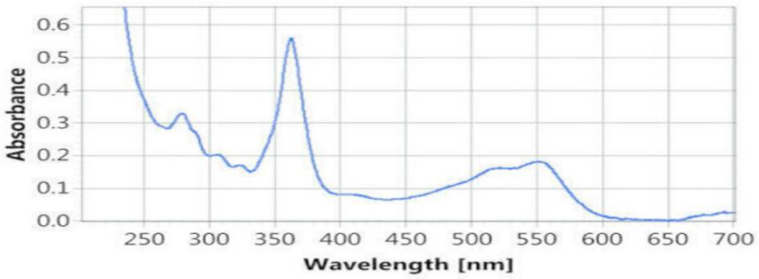

Fig.1: λ Max Of Vitamin B 12(Cyanocobalamin)

The absorption maximum (λmax) of Vitamin B12 was found to be 360 nm as shown in fig.1 Obtained peak were similar to literature reported peak. This value was selected for further analysis.

Standard calibration plot.

Absorbances of all the solution was measured at 360 nm against blank and calibration curve was constructed by taking concentration on x-axis and absorbance on y-axis.As shown in fig. 1.

Table 2: Standard calibration data of Vitamin B 12(Cyanocobalamin).

Sl. no	Concentration (µg/ml)	Absorbance ± SD*
1	**0**	**0**
2	**1**	**0.134±0.001**
3	**2**	**0.241±0.004**
4	**3**	**0.342±0.002**
5	**4**	**0.451±0.001**
6	**5**	**0.558±0.003**

7	**6**	**0.672±0.002**
8	**7**	**0.761±0.002**

***All the Values represents are mean of 3 readings (n=3) ±SD- Standard deviation.**

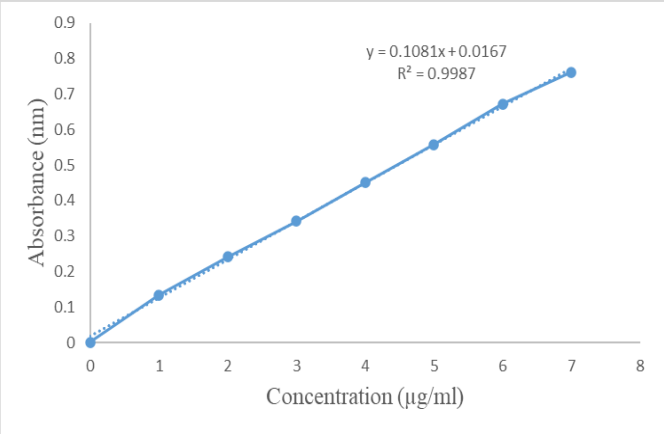

Fig 2: Standard calibration plot of Vitamin B12 (CYANOCOBALAMIN)

The drug solution of 1µg/ml - 7µg/ml was prepared using water and absorbance measured using UV spectrophotometer at the absorption maximum (λ max) 360nm. The obtained absorbancedata is plotted against the concentration of drug solution. Absorbance value remained linear and obeyed Beer's Lamberts law in the range of 0-7µg/ml with the slope value as y = 0.1081x+ 0.0167 and R^2 value of 0.9987. The values of the absorbance at different concentration(µg/ml) in IPA are given in the table No.7 and the standard plot is shown in fig 2.

COMPATIBILITY STUDIES USING FT-IR.

Compatibility of drug in physical mixture and formulation was analyzed by FT-IR. The prominent functional groups were observed and interpreted.

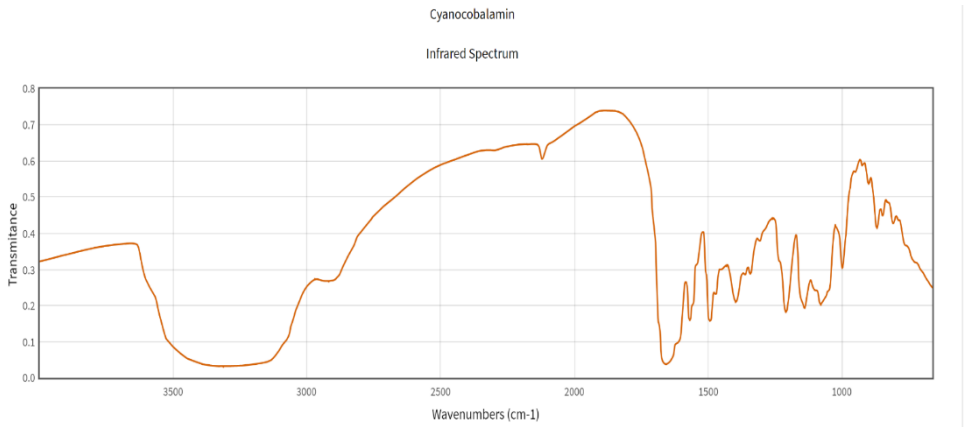

Fig 3 : Standard graph of FTIR spectrum Cyanocobalamin

The study of the inclusion complex between cyanocobalamin (Vitamin B12) and β-cyclodextrin (β-Cd) utilized Fourier Transform Infrared (FTIR) spectroscopy to analyse distinct functional groups across various formulations. Key findings include: the corrin ring absorption shifted slightly from 1545-1575 cm^{-1} to 1500-1573 cm^{-1}; C=O stretching vibrations changed from 1630-1675 cm^{-1} to 1635-1668 cm^{-1} for F-1 and F-2, and specifically to 1668

cm^{-1} for F-3; Co-C stretching remained stable at approximately 2130 cm^{-1}, varying slightly in formulations; O-H stretching vibrations shifted from 3200-3400 cm^{-1} to 3365-3412 cm^{-1}, indicating interactions with β-Cd; C-H vibrations for β-Cd were detected at 2928 cm^{-1}, with variations up to 2937 cm^{-1}; C-C stretching appeared consistently at 1157 cm^{-1}, and O-H bending was observed around 1029-1030 cm^{-1}. These shifts suggest the formation of an inclusion complex that may enhance the stability and solubility of vitamin B12, crucial for its bioavailability.

Table 3 : Inclusive complex of cyanocobalamin and Beta Cyclodextrin FTIR For Different Formulations:

Functional groups	Wavelength from 400 to 4000 cm^{-1}					
	Vitamin B12 (Cyanocobalamin)	**β-cyclodextrin**	**F-1**	**F-2**	**F-3**	**F-4**
Corrin ring	1545-1575	-	1500-1572	1500-1572	1502-1573	1500-1572
C=O stretching	1630-1675	-	1639-1668	1635-1668	1668	1668
Co-C stretching	2130	-	2133	2133	2135	2133
O-H stretching	3200-3400	3200-3400	3412	3394	3394	3365
C-H vibration	-	2928	2937	2931	2931	2928
C-C stretching	-	1157	1157	1157	1155	1157
OH bending vibration	-	1029	1030	1030	1030	1030

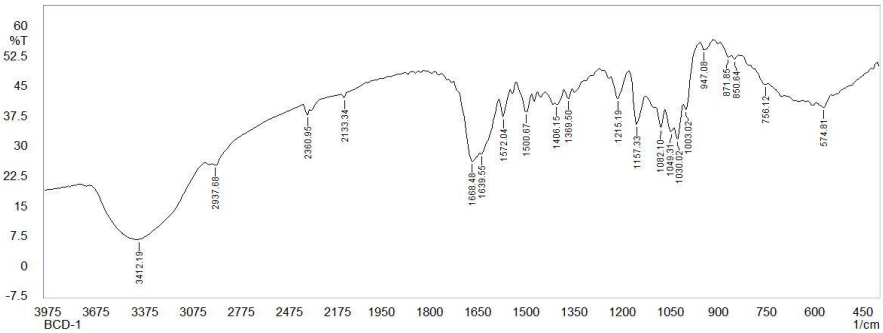

Fig 4: graph of FTIR spectrum for F1 representing different bands, identifying their functional groups of vitamin B12 and β-CD

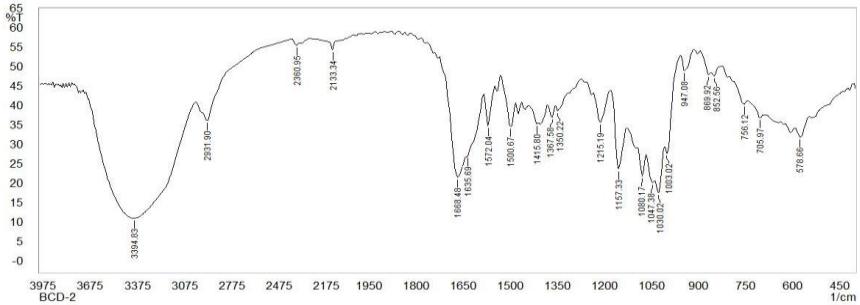

Fig 5: graph of FTIR spectrum for F2 representing different bands, identifying their functional groups of vitamin B 12 and β-CD

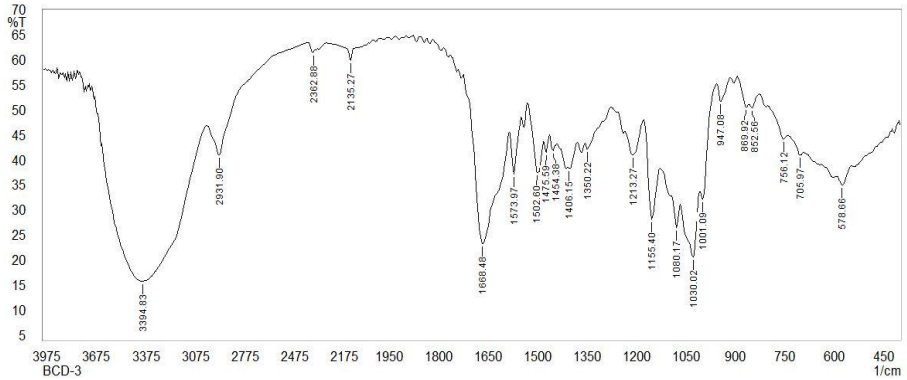

Fig 6: graph of FTIR spectrum for F3 representing different bands, identifying their functional groups of vitamin B 12 and β-CD

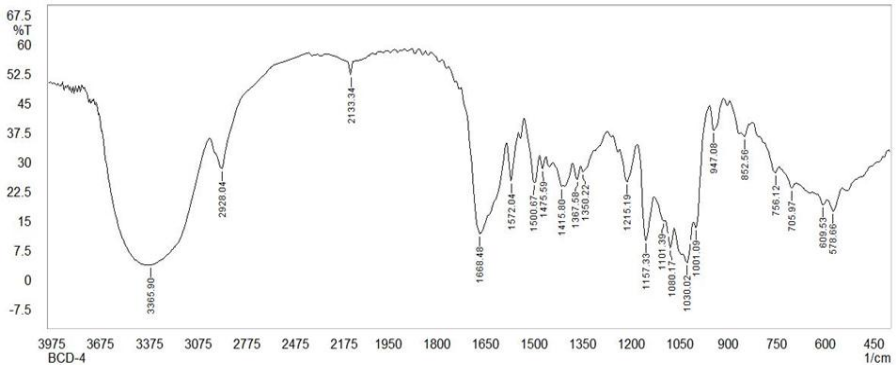

Fig 7: graph of FTIR spectrum for F4 representing different bands, identifying their functional groups of vitamin B 12 and β-CD

The FTIR analysis of the inclusion complex between cyanocobalamin (Vitamin B12) and hydroxypropyl-β-cyclodextrin (HP-β-Cyd) reveals significant interactions among functional groups. The corrin ring absorption peaks shifted slightly from 1575-1545 cm^{-1} to 1572-1573 cm^{-1} in formulations F-1 to F-4. C=O stretching for cyanocobalamin was detected between 1630 and 1675 cm^{-1}, shifting to 1033-1668 cm^{-1} with HP-β-Cyd, indicating interactions. Co-C stretching remained stable around 2130 cm^{-1}, while O-H stretching for cyanocobalamin (3200-3400 cm^{-1}) and HP-β-Cyd (3200-3600 cm^{-1}) suggested hydrogen bonding, with formulations showing values between 3394 and 3419 cm^{-1}. C-C stretching was absent in cyanocobalamin but present in HP-β-Cyd (900-1000 cm^{-1}), with formulation values from 947 to 1033 cm^{-1}. Overall, these findings confirm the formation of an inclusion complex that may enhance the stability and solubility of vitamin B12.

Table 4: Inclusive complex of cyanocobalamin and HP-Beta Cyclodextrin FTIR For Different Formulations:

Functional groups	Wavelength from 400 to 4000 cm^{-1}					
	Vitamin B12 (Cyanocobalamin)	**HP-β-cyclodextrin**	**F-1**	**F-2**	**F-3**	**F-4**
Corrin ring	1575-1545	-	1572	1573	1573	1572
C=O stretching	1630-1675	1000-1200	1033-1668	1033-1668	1033-1670	1035-1666
Co-C strectching	2130	-	2135	2135	2137	2135
O-H stretching	3200-3400	3200-3600	3396	3394	3419	3396
C-C strectching	-	900-1000	949-1033	949-1033	947-1033	947-1035

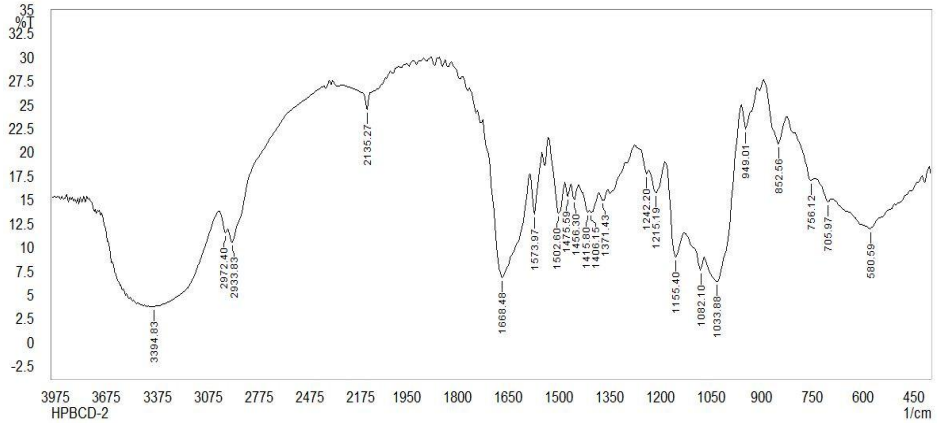

Fig 8: graph of FTIR spectrum for F1 representing different bands, identifying their functional groups of vitamin B 12 and HP- β-CD

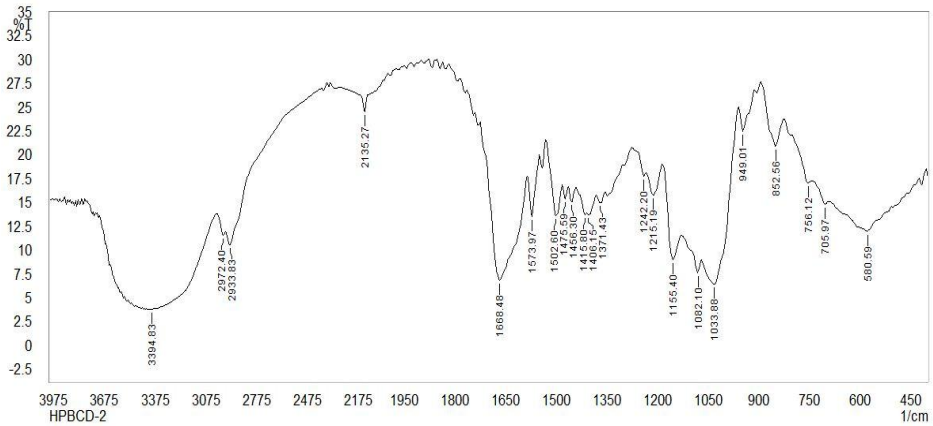

Fig 9: graph of FTIR spectrum for F2 representing different bands, identifying their functional groups of vitamin B 12 and HP- β-CD

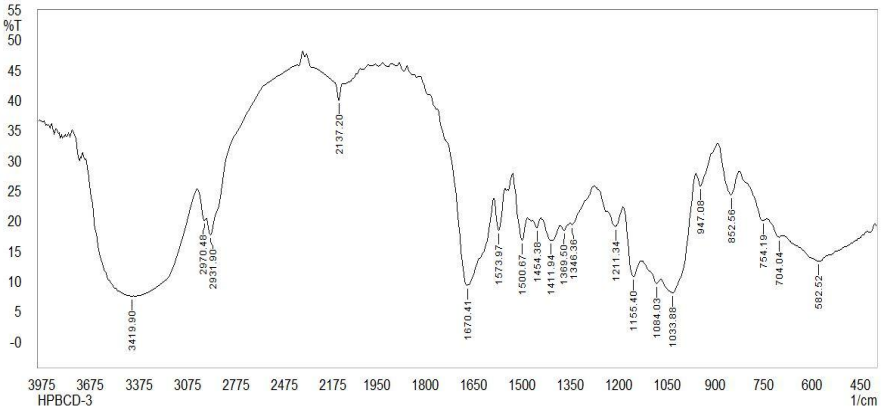

Fig 10: graph of FTIR spectrum for F3 representing different bands, identifying their functional groups of vitamin B 12 and HP- β-CD

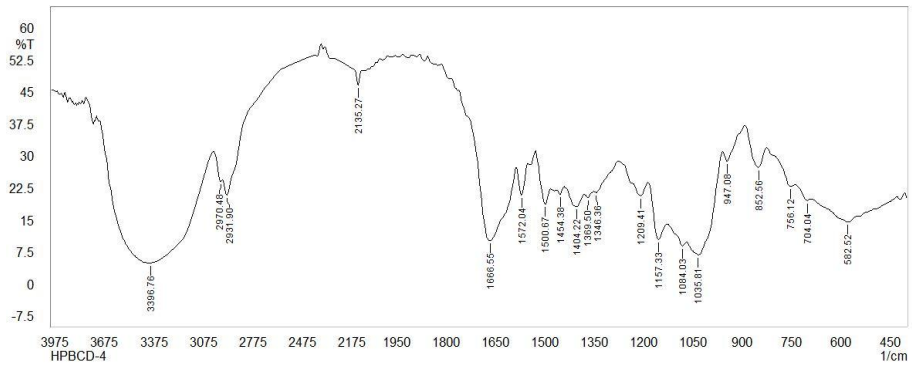

Fig 11: graph of FTIR spectrum for F4 representing different bands, identifying their functional groups of vitamin B 12 and HP- β-CD

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The DSC analysis of cyanocobalamin complexes shows that pure cyanocobalamin has an onset temperature of **250°C** and an enthalpy change of **-200 mJ**. Formulations F1 to F4 vary slightly, with F1 at **252°C** (-59 mJ), F2 at **256°C** (-72 mJ), F3 at **249°C** (-66 mJ), and F4 at **254°C** (-101 mJ), indicating altered stability due to complexation with β-cyclodextrin. In contrast, the complex with hydroxypropyl-β-cyclodextrin (HP-β-Cyd) enhances thermal properties, where cyanocobalamin again shows an onset temperature of **250°C**, but F1 demonstrates improved stability with an onset of **249°C** and an enthalpy change of **-290 mJ**. F2 maintains a similar onset but has a lower enthalpy change of **-101 mJ**, while F3 and F4 show lower stability with enthalpy changes of **-76 mJ** and **-78 mJ**, respectively.

Table 5: Interpretation of DSC thermogram of inclusive complex of cyanocobalamin and beta cd.

Sample	Tp onset ^{O}C	Peak ^{O}C	Tp End ^{O}C	ΔH_f mJ
cyanocobalamin	250	300	260	-200
F1	252	259	267	-59
F2	256	261	267	-72
F3	249	260	270	-66
F4	254	261	270	-101

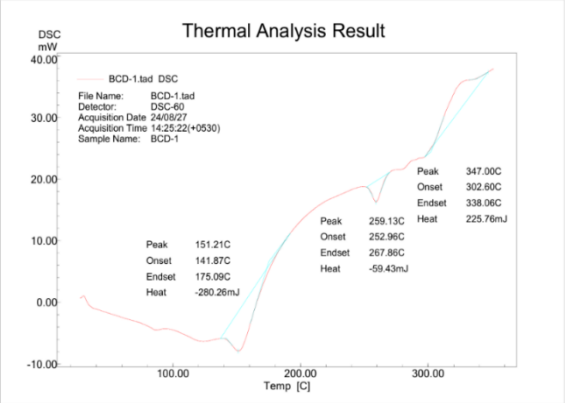

Fig 12: DSC thermogram of F1 Showing onset at 252 ^{O}C, peak at 259 ^{O}C, endset at 267 ^{O}C and ΔH_f at -59mJ

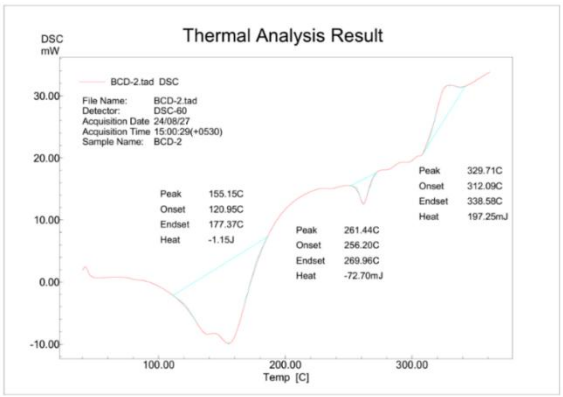

Fig 13: DSC thermogram of F2 Showing onset at 256 ^{O}C, peak at 261 ^{O}C, endset at 269 ^{O}C and ΔH_f at -72 mJ

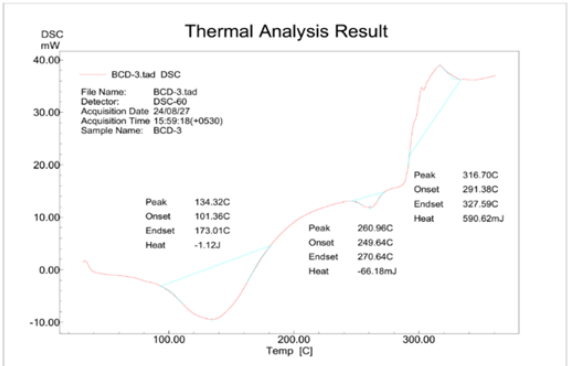

Fig 14: DSC thermogram of F3 Showing onset at 249 ^{O}C, peak at 260 ^{O}C, endset at 270 ^{O}C and ΔH_f at -66mJ

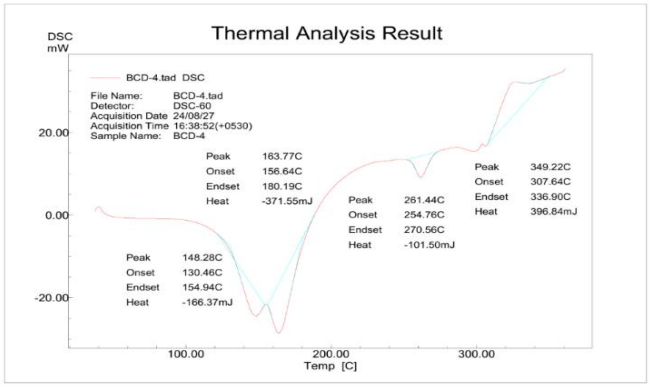

Fig 15: DSC thermogram of F4 Showing onset at 254 ^{O}C, peak at 261 ^{O}C, endset at 270 ^{O}C and ΔH_f at -101mJ

Table 6: interpretation of DSC thermogram of inclusive complex of cyanocobalamin and Hp-beta cd.

Sample	Tp onset ^{O}C	Peak ^{O}C	Tp End ^{O}C	ΔH_f mJ
cyanocobalamin	250	300	260	-200
F1	249	257	278	-290
F2	249	257	271	-101
F3	247	255	263	-76
F4	250	256	266	-78

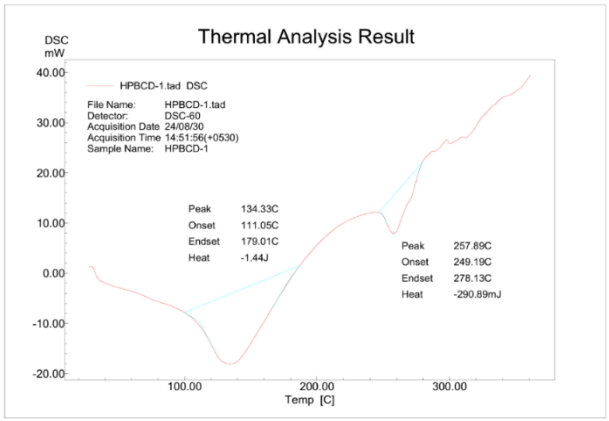

Fig 16: DSC thermogram of F1 Showing onset at 249 ^{O}C, peak at 257 ^{O}C, endset at 278 ^{O}C and ΔH_f at -290mJ

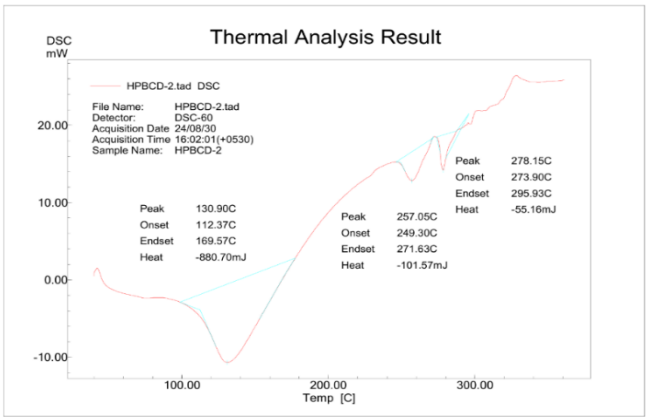

Fig 17: DSC thermogram of F2 Showing onset at 249 ^{O}C, peak at 257 ^{O}C, endset at 271 ^{O}C and ΔH_f at 101mJ

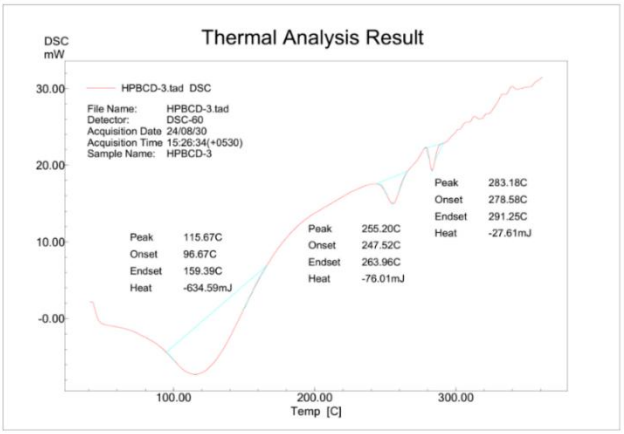

Fig 18: DSC thermogram of F3 Showing onset at 247 ^{O}C, peak at 255 ^{O}C, endset at 263 ^{O}C and ΔH_f at -76mJ

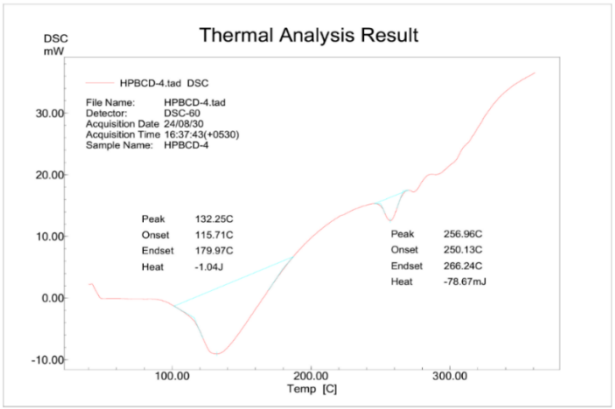

Fig 19 : DSC thermogram of F1 Showing onset at 250 ^{O}C, peak at 256 ^{O}C, endset at 266 ^{O}C and ΔH_f at -76 mJ

***IN VITRO* DRUG RELEASE STUDY**

DISSOLUTION OF VITAMIN B 12 AND BETA CYCLODEXTRIN:

The %CDR data for vitamin B12 with β-cyclodextrin from formulations F1, F2, F3, and F4 shows varying release profiles. At 1-minute, cumulative releases were 45.6% (F1), 72.0% (F2), 67.7% (F3), and 88.9% (F4). After 2 minutes, these increased to 50.8% (F1), 82.9% (F2), 92.7% (F3), and 95.8% (F4). By 3 minutes, F2, F3, and F4

nearly reached complete release at 98.7%, 99.6%, and 100.6%. At 5 minutes, F1 was at 99.8%, while F2 and F4 slightly exceeded 100% at 102.1%. This highlights the enhanced release profile of vitamin B12 with β-cyclodextrin.

Table 7 : Percentage Cumulative Drug Release Data Of Vitamin B 12 With Beta Cd Powder From Different Formulations

Time (min)	Cumulative drug release %			
	F1	**F2**	**F3**	**F4**
1	45.6	72.0	67.7	88.9
2	50.8	82.9	92.7	95.8
3	72.5	98.7	99.6	100.6
4	80.9	99.9	100.5	101.5
5	99.8	102.1	101.6	102.1

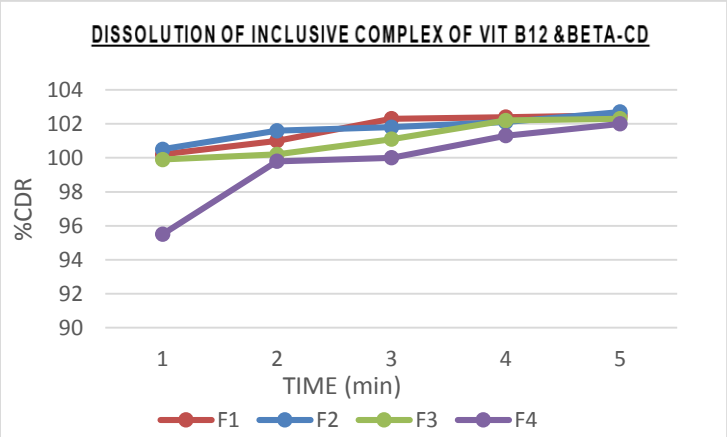

Fig 20: graph of percentage cumulative drug release data of vitamin b 12 with beta cd powder from different formulations

DISSOLUTION OF VITAMIN B 12 AND HP-BETA CYCLODEXTRIN:

The cumulative drug release data for vitamin B12 with hydroxypropyl-β-cyclodextrin (HP-β-Cd) from formulations F1, F2, F3, and F4 shows strong release percentages over time. At 1 minute, releases were 100.2% (F1), 100.5% (F2), 99.9% (F3), and 95.5% (F4). After 2 minutes, these increased to 101% (F1), 101.6% (F2), 100.2% (F3), and 99.8% (F4). By 5 minutes, the cumulative releases reached 102.5% (F1), 102.7% (F2), 102.3% (F3), and 102.0% (F4), indicating a robust release profile for all formulations.

Table 8: Percentage cumulative drug release data of Vitamin B 12 With Hp-Beta Cd powder from formulations

Time (min)	Cumulative % drug release			
	F1	**F2**	**F3**	**F4**
1	100.2	100.5	99.9	95.5
2	101	101.6	100.2	99.8
3	102.3	101.8	101.1	100.0
4	102.4	102.1	102.2	101.3
5	102.5	102.7	102.3	102.0

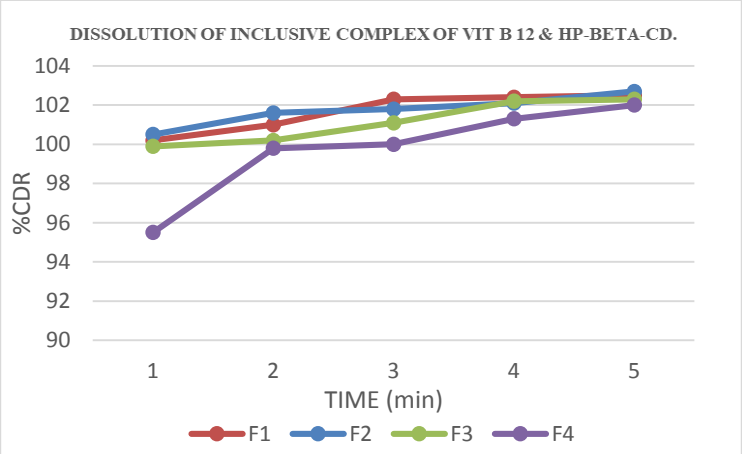

Fig 21: Graph of Percentage cumulative drug release data of Vitamin B 12 With HP- Beta Cd powder from different formulations.

MICROMERITIC STUDY

a. Angle of Repose(θ):

The values of the angle of repose of inclusive complex of vitamin B 12 and cyclodextrins were in the range of 30–400, which indicates the passable flow property of all the formulations. Values of the angle of repose are shown in Table 14.

b. Carr's Index (Ci):

The Carr's index values for inclusive complex of vitamin B 12 and cyclodextrins were in the range 21-25% in the beta-cd, which lies between the passable properties, and in the Hp-beta-cd, between 11–15%, which shows good flow properties. Values of the carr's index are shown in Table 14.

c. Hausner's ratio:

The Hausner's ratio values for inclusive complex of vitamin B 12 and cyclodextrins were found to be in the range 1.26 to 1.27 in beta-cd; these values are within the range of 1.26-1.34 which indicates powder exhibited passable flow properties. And 1.26-2.27 in the Hp-beta-cd; these values are within the range of 1.12-1.18, which indicated that the powder mixture exhibited good flow properties. An obtained value of Hausner's ratio is shown in Table 14.

d. Bulk Density and Tapped Density: The bulk and tapped density measurements of vitamin B12 formulations with beta-cyclodextrin (β-CD) and hydroxypropyl-beta-cyclodextrin (HP-β-CD) show notable differences. Formulations with β-CD had bulk densities ranging from 0.233 to 0.253 g/ml and tapped densities from 0.307 to 0.322 g/ml, indicating lower packing efficiency. In contrast, HP-β-CD formulations exhibited higher bulk densities of 0.416 to 0.465 g/ml and tapped densities of 0.487 to 0.540 g/ml, suggesting improved packing characteristics.

Table 9: Micromeritic properties of Vitamin B 12 Inclusive Complex of vitamin B12 and cyclodextrins.

Formulation code	Angle of repose ($_{\theta}O$)	Bulk Density (gm/ml)	TappedDensity (gm/ml)	Carr's index (%)	HausnerRatio
With Beta cyclodextrin					
F1	38.65±0.24	0.253±0.02	0.322±0.01	21.42±0.4	1.27±0.02
F2	37.95±0.31	0.243±0.01	0.307±0.01	20.84±0.2	1.26±0.04
F3	39.35±0.27	0.240±0.01	0.307±0.03	21.82±0.5	1.27±0.02
F4	37.88±0.24	0.233±0.01	0.323±0.01	20.66±0.01	1.26±0.08
With HP-Beta cyclodextrin					
F1	32.61±0.18	0.465±0.02	0.540±0.02	13.83±0.6	1.16±0.03
F2	30.54±0.35	0.444±0.03	0.513±0.02	13.33±0.3	1.15±0.02
F3	31.38±0.23	0.416±0.02	0.487±0.01	14.57±0.4	1.17±0.02
F4	33.95±0.31	0.434±0.01	0.490±0.01	14.99±0.2	1.14±0.04

***All the Values represents are mean of 3 readings (n=3) ±S. D**

STABILITY STUDIES

The stability studies of vitamin B12 with β-cyclodextrin over 30 days showed initial drug content percentages of **100.3%** (F1), **101.9%** (F2), **102.5%** (F3), and **101.5%** (F4). After 15 days, these values decreased slightly to **99.7%**, **100.1%**, **100.4%**, and **100.2%**, respectively, and by day 30, they further declined to **98.3%**, **99.9%**, **98%**, and **99%**.Incomparison, with hydroxypropyl-β-cyclodextrin(HP-β-Cd),initial values were 102.1% (F1), 101% (F2), 102.2% (F3), and 100.1% (F4). After 15 days, they dropped to 100.1%, 99.3%, 99%, and 98.2%, and by day 30, to 98%, 97.9%, 98.2%, and 97.3%. All results indicate stable formulations throughout the study period.

Table 10 : Stability studies of vitamin B12 with beta cyclodextrin inclusive complex product.

Time (days)	Drug content (%)			
	F1	**F2**	**F3**	**F4**
0	100.3	101.9	102.5	101.5
15	99.7	100.1	100.4	100.2

30	98.3	99.9	98	99

***All the Values represents are mean of 3 readings (n=3)**

Table 11 : Stability studies of vitamin B12 with HP-beta cyclodextrin inclusive complex product.

Time (days)	Drug content (%)			
	F1	**F2**	**F3**	**F4**
0	102.1	101	102.2	100.1
15	100.1	99.3	99	98.2
30	98	97.9	98.2	97.3

***All the Values represents are mean of 3 readings (n=3)**

IV. Conclusion

In conclusion, the study recommends beta-cyclodextrin (β-CD) as the preferred excipient for formulating stabilized vitamin B12 complexes due to its superior solubility, compatibility, and enhanced release profile. This formulation approach holds promise for stability in particular photostability.

References

[1]. Wdowik T, Gryko D. C–C Bond Forming Reactions Enabled by Vitamin B12─ Opportunities and Challenges. ACS Catalysis. 2022 May 17;12(11):6517-31. https://doi.org/10.1021/acscatal.2c01596.

[2]. Qadeer K, Arsalan A, Ahmad I, Fatima K, Anwar Z, Ahmed S, Mahmud S. Photochemical interaction of cyanocobalamin and hydroxocobalamin with cysteine. Journal of Molecular Structure. 2021 Mar 15; 1228:129441. https://doi.org/10.1016/j.molstruc.2020.129441

[3]. Juzeniene A, Nizauskaite Z. Photodegradation of cobalamins in aqueous solutions and in human blood. Journal of Photochemistry and Photobiology B: Biology. 2013 May 5; 122:7-14. https://doi.org/10.1016/j.jphotobiol.2013.03.001

[4]. Carneiro SB, Costa Duarte FÍ, Heimfarth L, Siqueira Quintans JD, Quintans-Júnior LJ, Veiga Júnior VF, Neves de Lima ÁA. Cyclodextrin–drug inclusion complexes: In vivo and in vitro approaches. International journal of molecular sciences. 2019 Feb 2;20(3):642. https://doi.org/10.3390/ijms20030642

[5]. Iacovino R, Rapuano F, Caso JV, Russo A, Lavorgna M, Russo C, Isidori M, Russo L, Malgieri G, Isernia C. β-Cyclodextrin inclusion complex to improve physicochemical properties of pipemidic acid: Characterization and bioactivity evaluation. International journal of molecular sciences. 2013 Jun 25;14(7):13022-41. https://doi.org/10.3390/ijms140713022.

[6]. Erdő s M, Hartkamp R, Vlugt TJ, Moultos OA. Inclusion complexation of organic micropollutants with β-cyclodextrin. The Journal of Physical Chemistry B. 2020 Jan 24;124(7):1218-28. https://doi.org/10.1021/acs.jpcb.9b10122

[7]. Juzeniene A, Nizauskaite Z. Photodegradation of cobalamins in aqueous solutions and in human blood. Journal of Photochemistry and Photobiology B: Biology. 2013 May 5;122:7-14. https://doi.org/10.1016/j.jphotobiol.2013.03.001.

[8]. Kiran Q, Tania M, Zufi S, Iqbal A. Photodegradation of cyanocobalamin in the presence of ascorbic acid. emr-195283

[9]. Ahmad I, Hussain W, Fareedi AA. Photolysis of cyanocobalamin in aqueous solution. Journal of pharmaceutical and biomedical analysis. 1992 Jan 1;10(1):9-15. https://doi.org/10.1016/0731-7085(92)80004-7.

[10]. Ahmad I, Ahmed S, Anwar Z, Sheraz MA, Sikorski M. Photostability and photostabilization of drugs and drug products. International Journal of Photoenergy. 2016 May 18;2016. https://doi.org/10.1155/2016/8135608.

[11]. Giedyk M, Gryko D. Vitamin B12: An efficient cobalt catalyst for sustainable generation of radical species. Chem Catalysis. 2022 Jul 21;2(7):1534-48. https://doi.org/10.1016/j.checat.2022.05.004.

[12]. Lee W, Lee YB, Huh MH, Choi JK. Determination of the chemical stability of cyanocobalamin in medical food by a validated immunoaffinity column-linked HPLC method. Journal of Food Quality. 2022 Jan 12;2022:1-8. https://doi.org/10.1155/2022/1619936

[13]. Wang M, Schuster K, Asam S, Rychlik M. Challenges in the determination of total vitamin B12 by cyanidation conversion: insights from stable isotope dilution assays. Analytical and Bioanalytical Chemistry. 2023 Sep;415(23):5797-807. https://doi.org/10.1007/s00216-023-04860-y.

[14]. Rampazzo G, Zironi E, Pagliuca G, Gazzotti T. Analysis of Cobalamin (Vit B12) in Ripened Cheese by Ultra-High-Performance Liquid Chromatography Coupled with Mass Spectrometry. Foods. 2022 Sep 7;11(18):2745. https://doi.org/10.3390/foods11182745.

[15]. Devi S, Pasanna RM, Shamshuddin Z, Bhat K, Sivadas A, Mandal AK, Kurpad AV. Measuring vitamin B-12 bioavailability with [13C]- cyanocobalamin in humans. The American Journal of Clinical Nutrition. 2020 Dec;112(6):1504-15. https://doi.org/10.1093/ajcn/nqaa221.

[16]. Akbari N, Assadpour E, Kharazmi MS, Jafari SM. Encapsulation of Vitamin B12 by Complex Coacervation of Whey Protein Concentrate–Pectin; Optimization and Characterization. Molecules [Internet]. 2022 Sep 19;27(18):6130. https://doi.org/10.3390/molecules27186130

[17]. Lee W, Lee YB, Huh MH, Choi JK. Determination of the chemical stability of cyanocobalamin in medical food by a validated immunoaffinity column-linked HPLC method. Journal of Food Quality. 2022 Jan 12; 2022:1-8. https://doi.org/10.1155/2022/1619936.

[18]. Ahmad I, Qadeer K, Zahid S, Sheraz MA, Ismail T, Hussain W, Ansari IA. Effect of ascorbic acid on the degradation of cyanocobalamin and hydroxocobalamin in aqueous solution: a kinetic study. AAPS PharmSciTech. 2014 Oct; 15:1324 -33. https://doi.org/10.1208%2Fs12249-014-0160-5

[19]. Barman BK, Rajbanshi B, Yasmin A, Roy MN. Exploring inclusion complexes of ionic liquids with α-and β-cyclodextrin by NMR, IR, mass, density, viscosity, surface tension and conductance study. Journal of Molecular Structure. 2018 May 5; 1159:205 -15. https://doi.org/10.1016/j.molstruc.2018.01.062

[20]. Bodur S, Erarpat S, Balçık U, Bakırdere S. A rapid, sensitive and accurate determination of cobalamin with double monitoring system: HPLC-UV and HPLC-ICP-OES. Food Chemistry. 2021 Mar 15; 340:127945. https://doi.org/10.1016/j.foodchem.2020.127945

[21]. Xu X, Peng S, Bao G, Zhang H, Yin C. β-cyclodextrin inclusion complexes with vitamin A and its esters: A comparative experimental and molecular modeling study. Journal of Molecular Structure. 2021 Jan 5; 1223:129001. https://doi.org/10.1016/j.molstruc.2020.129001

[22]. Cid-Samamed A, Rakmai J, Mejuto JC, Simal-Gandara J, Astray G. Cyclodextrins inclusion complex: Preparation methods, analytical techniques and food industry applications. Food Chemistry. 2022 Aug 1; 384:132467. https://doi.org/10.1016/j.foodchem.2022.132467.

[23]. Mazzocato MC, Thomazini M, Favaro-Trindade CS. Improving stability of vitamin B12 (Cyanocobalamin) using microencapsulation by spray chilling technique. Food Research International. 2019 Dec 1; 126:108663. https://doi.org/10.1016/j.foodres.2019.108663.

[24]. Carlan IC, Estevinho BN, Rocha F. Study of microencapsulation and controlled release of modified chitosan microparticles containing vitamin B12. Powder Technology. 2017 Aug 1; 318:162-9. https://doi.org/10.1016/j.powtec.2017.05.041.

[25]. Kumar SS, Chouhan RS, Thakur MS. Trends in analysis of vitamin B12. Analytical biochemistry. 2010 Mar 15;398(2):139 -49. http://dx.doi.org/10.1016/j.ab.2009.06.041.

[26]. Estevinho BN, Carlan I, Blaga A, Rocha F. Soluble vitamins (vitamin B12 and vitamin C) microencapsulated with different biopolymers by a spray drying process. Powder Technology. 2016 Feb 1; 289:71-8. https://doi.org/10.1016/j.powtec.2015.11.019

[27]. Aman W, Thoma K. ICH guideline for photostability testing: aspects and directions for use. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2003 Dec 1;58(12):877-80.

[28]. Rajbanshi B, Dutta A, Mahato B, Roy D, Maiti DK, Bhattacharyya S, Roy MN. Study to explore host guest inclusion complexes of vitamin B1 with CD molecules for enhancing stability and innovative application in biological system. Journal of Molecular Liquids. 2020 Jan 15;298:111952.

[29]. Temova Rakuša Ž, Roškar R, Hickey N, Geremia S. Vitamin B12 in foods, food supplements, and medicines—a review of its role and properties with a focus on its stability. Molecules. 2022 Dec 28;28(1):240.

[30]. Saha S, Roy A, Roy K, Roy MN. Study to explore the mechanism to form inclusion complexes of β-cyclodextrin with vitamin molecules. Scientific reports. 2016 Oct 20;6(1):1-2.

[31]. Bakirova R, Nukhuly A, Iskineyeva A, Fazylov S, Burkeyev M, Mustafayeva A, Minayeva Y, Sarsenbekova A. Obtaining and Investigation of the β- Cyclodextrin Inclusion Complex with Vitamin D3 Oil Solution. Scientifica. 2020;2020(1):6148939.

[32]. Aiassa V, Garnero C, Zoppi A, Longhi MR. Cyclodextrins and their derivatives as drug stability modifiers. Pharmaceuticals. 2023 Jul 28;16(8):1074.

[33]. Monajjemzadeh F, Ebrahimi F, Zakeri-Milani P, Valizadeh H. Effects of formulation variables and storage conditions on light protected vitamin B12 mixed parenteral formulations. Advanced Pharmaceutical Bulletin. 2014 Dec;4(4):329.

[34]. Taraszka KS, Chen E, Metzger T, Chance MR. Identification of structural markers for vitamin B12 and other corrinoid derivatives in solution using FTIR spectroscopy. Biochemistry. 1991 Feb 1;30(5):1222-7.

[35]. David I, Orboi MD, Simandi MD, Chirilă CA, Megyesi CI, Rădulescu L, Drăghia LP, Lukinich-Gruia AT, Muntean C, Hădărugă DI, Hădărugă NG. Fatty acid profile of Romanian's common bean (Phaseolus vulgaris L.) lipid fractions and their complexation ability by β-cyclodextrin. PLoS One. 2019 Nov 22;14(11):e0225474.

[36]. Sambasevam KP, Mohamad S, Sarih NM, Ismail NA. Synthesis and characterization of the inclusion complex of β-cyclodextrin and azomethine. International journal of molecular sciences. 2013 Feb 7;14(2):3671-82.

[37]. Salimi A, Zadeh BS, Moghimipour E. Preparation and characterization of cyanocobalamin (Vit B12) microemulsion properties and structure for topical and transdermal application. Iranian journal of basic medical sciences. 2013 Jul;16(7):865.

[38]. Ma'ali A, Naseef H, Qurt M, Abukhalil AD, Rabba AK, Sabri I. The Preparation and Evaluation of Cyanocobalamin Mucoadhesive Sublingual Tablets. Pharmaceuticals. 2023 Oct 4;16(10):1412.

[39]. Sarheed OM, Ramesh KV, Shah FA. In vitro evaluation of dissolution profile of two commercially available folic acid preparations. Int J pharm pharm Sci. 2015;7(3):473-5.

[40]. Fang Huan FH, Kang Jie KJ, Zhang DaWei ZD. Microbial production of vitamin B12: a review and future perspectives.

[41]. Jansen EH, Beekhof PK. Stability of folate and vitamin B12 in human serum after long- term storage: a follow- up after 13 years. Journal of nutrition and metabolism. 2018;2018(1):9834181.

[42]. Ata F, Bilal AB, Javed S, Chaudhry HS, Sharma R, Malik RF, Choudry H, Kartha AB. Optic neuropathy as a presenting feature of vitamin B-12 deficiency: a systematic review of literature and a case report. Annals of Medicine and Surgery. 2020 Dec 1;60:316-22.

[43]. Monajjemzadeh F, Ebrahimi F, Zakeri-Milani P, Valizadeh H. Effects of formulation variables and storage conditions on light protected vitamin B12 mixed parenteral formulations. Advanced Pharmaceutical Bulletin. 2014 Dec;4(4):329.

[44]. Salimi A, Zadeh BS, Moghimipour E. Preparation and characterization of cyanocobalamin (Vit B12) microemulsion properties and structure for topical and transdermal application. Iranian journal of basic medical sciences. 2013 Jul;16(7):865.

[45]. Estevinho BN, Carlan I, Blaga A, Rocha F. Soluble vitamins (vitamin B12 and vitamin C) microencapsulated with different biopolymers by a spray drying process. Powder Technology. 2016 Feb 1;289:71-8.