بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate studies

A Study of Cobalamin Complexes with some Amino Acids

دراسة معقدات الكوبالامين مع بعض الأحماض الأمينية

A
Thesissubmittedinpartialfullfortherequirementsofth e Degree of Master in chemistry

By:

WesamAbdalgfor Mohamed Fadulallah

(B.Sc Honors, Chemistry)

Supervisor : Dr. Prof : Mohammed Adam Abbo

Feb 2022

إستهلال

بسم الله الرحمن الرحيم

قال تعالى :

﴿يؤتي الحكمة من يشاء ومن يؤت الحكمة فقد أوتي خيراً كثيراً وما يذكر إلا أولو الألباب ﴾ (269)

سورةالبقرة (269)

Dedication

To:

Mylovely parents with special feeling of gratitude,

To my brothers ,

I am truly thankful for having youin my life.

Acknowledgments

first and foremost , praise and thanks to Allah, Almighty ,my creator, my strong pillar , my source of inspiration ,wisdom ,knowledge and understanding ,for being the source of my strength through this research.

My deep and sincere gratitude to my research supervisor ,Prof. Mohmmed Adam Abbo, for his dynamism, vision ,sincerity and motivation .

It was a great opportunity to learn and under his guidance.

I am very grateful forthe teaching and technician staff of chemistry department in Sudan University of Science and Technology for their help ,especial thanks to Mr.Majed .

My thanks should be extended to the staff of Shanghai-Sudan pharmaceutical CO.,Ltd , Quality control department for allow me to work in their labs.

My thanks to Mr. Akram at Alneleen University department of biochemistry .

Sincere thanks extended to my dearest colleagues and my beloved friends who have been so supportive along the way.

Last but not least , I would like to thanks my family for supporting me throughout my life.

ABSTRAC

The aimof this study was to preparecobalamin and amino acids complexes to investigate the possibility of designing a drug and study its biological and chemical activity (behaviour).

The study also was aimed to propose the appropriate conditions for complex formation.

The experiments were carried by replacing water molecules asweak ligands ligand in the upper part (β) of the cobalamin by amino acids.

Two types of amino acids with different donor atoms were used: alanine and cysteine.

All solutions were prepared and placed in a water path at 40 Cfor one hour and the pH was measured.

The pH of aquacobalamin was found to be 5.4 .

The pH of alanine-cobalamin complex and cysteine-cobalamin complex was adjusted at pH=2 and pH=12.4) .

the (UV-vis) spectrum of resulting solutions was recorded at rang of 200-800 nm,the spectrum showed that alanine-cobalamin complex which prepared in acidic medium gave small change compared to aqua cobalamin but the complex which prepared in alkaline medium did not show a change, while the cysteine-cobalamin complex at pH =12.4 showed a clear change .

The optical activity (α) was determined by a Polarimeter .

The aquacobalamin and Alanine-cobalamin complex at pH=2 gavevalue with positive sign (+ 0.057 and + 0.031 respectively),while cysteine-cobalamin complex at pH =12.4 gave a negative value (- 0.100) .

المستخلص

تهدف هذه الدراسة لتحضير معقدات بين الكوبالمين والاحماض الامينية لدراسة امكانية تحضير دواء ودراسة نشاطه الكيميائي والبيولوجي (السلوك) .

الدراسة هدفت أيضاً لمعرفة الظروف الملائمة لتكوين المعقد .

تمت التجربة باستبدال جزيئات الماء اللواقط الضعيفة الموجودة في الجزء الأعلى من الكوبالمين بواسطة الأحماض الامينية.
تم استخدام نوعين مختلفين من الأحماض الأمينية بذرات مانحة مختلفة وهما : الألنين والسستين.
كل المحاليل حضرت ووضعت في حمام عند درجة حرارة 40 درجة مئوية لمدة ساعة واحدة، وتم قياس الرقم الهيدروجيني، وجد أنه يساوي 5.4 لمحلول أكواكوبالمين .
الرقم الهيدروجيني لمعقدات الكوبالمين-ألنين والكوبالمين-سستين تم ضبطها في وسط عند (رقم هيدروجيني = 2 ورقم هيدروجيني = 12.4) .
تم استخدام جهاز مطيافية الاشعة فوق البنفسجيةلقياس الطول الموجي في المدى200-800نانو ميتر، النتائج المتحصل عليها وضحت أن معقد كوبالمين-ألنين المحضر في وسط حمضي اعطى تغير بسيطمقارنة بمحلولأكواكوبالمين اما المحضر في وسط قاعدي لم يعطي أي تغيير، في حين أن معقد كوبالمين-سستينعند الرقم الهيدروجيني 12.4 اعطى تغير واضح .
الدوران النوعي ايضا تم تعيينه باستخدام جهاز البولارميتر، محلول أكواكوبالمين ومعقد كوبالمين-ألنين أعطيا قيمة موجبة (+ 0,057 و + 0,031 على التوالي) .
في حين أن معقد الكوبالمين- سستين أعطى قيمة سالبة (– 0,100)

Table of Contents

List of Tables

List of Figures:

List of Abbreviations

Abbreviation	**Meaning and concept**
Cbls	Cobalamins
Cbi	Cobinamides
CNCbl	Cyanocobalamin
MeCbl	Methylcobalamin
AdoCbl	Adenosylcobalamin
H_2OCbl	Aquacobalamin
HC	Haptocorrin
IF	Intrinsic factor
TC	Trans cobalamin
DTPA	diethylenetriamine- N,N,N',N",N"'-pentaacetic acid
TTHA	triethylenetetramine-N,N,N',N",N"',N"'-hexaacetic acid
SAM	S.adenosyl methionine
MTA	MethyleTransferase
MDDR	MDL Drug Data Report
CMC	Comprehensive Medicinal Chemistry Database
ACD	Available Chemicals Directory

Chapter One

Introduction and literature review

1. Introduction and Literature review

1.1 General Introduction:

The transition metal ions which are available to a biological system are those from scandium to copper and from yttrium to molybdenum in the periodic table (manganese, iron, cobalt, copper and molybdenum) are of major importance, while vanadium , chromium and niobium are of much less interest.

In recent years transition metals-amino acids complexes have received much attention because they proved to be useful antibacterial agents (Rusu,*et al*.,2009) .

Twenty natural amino acids comprise the building blocks of proteins, which are chemical species indispensable to perform a large number of biological functions.

Complexes of transition metals with amino acids in proteins and peptides are utilized in numerous biological processes, such as oxygen conveyer, electron transfer and oxidation (Stanila,*et al*.,2007)

Metals are thought to play structural and chemical roles as cofactors of more than one-third of the proteome of most organisms,and for regulation of cellular function (Rosenzweig,2002) .

1.1.1 Cobalt:

Cobalt is a transition metal that occupies a position in the periodic table between iron and nickel.

It has two naturally occurring oxidation states (Co^{2+} and Co^{3+}) but can exhibit oxidation states from ± I to +IV .

This metal is rare in comparison with all known essential trace elements except cadmium and tungsten: 0.0025% (w/w) in the Earth's crust and 4×10^{-8} % and (w/v) in sea water.

Radioactive cobalt[^{60}Co] which is produced by thermal neutron bombardment of the natural isotope[^{59}Co] has a half-life of 5.3 years.

(Budavari ,*et al*.,1989).

It is used as a concentrated source of radiation in cancer therapy , food sterilization , radioactive tracer in biological and in industrial applications.

Because cobalt has characteristic spectra correlated with structural properties (such as observed co-ordination numbers for(Co^{2+} and Co^{3+}), it has also served as a spectroscopic probe in metalloenzymes (Maret and Vallee,1993).

Non-corrin cobalt is receiving increased interest not only in bioinorganic chemistry but also in biotechnology, and its availability and remarkable chemical versatility makes cobalt an invaluable catalyst in the chemical industry (e.g. hydro-formylation) (Pino,*etal*.,1977).

1.1.2 Coordination chemistry of Co^{+3}:

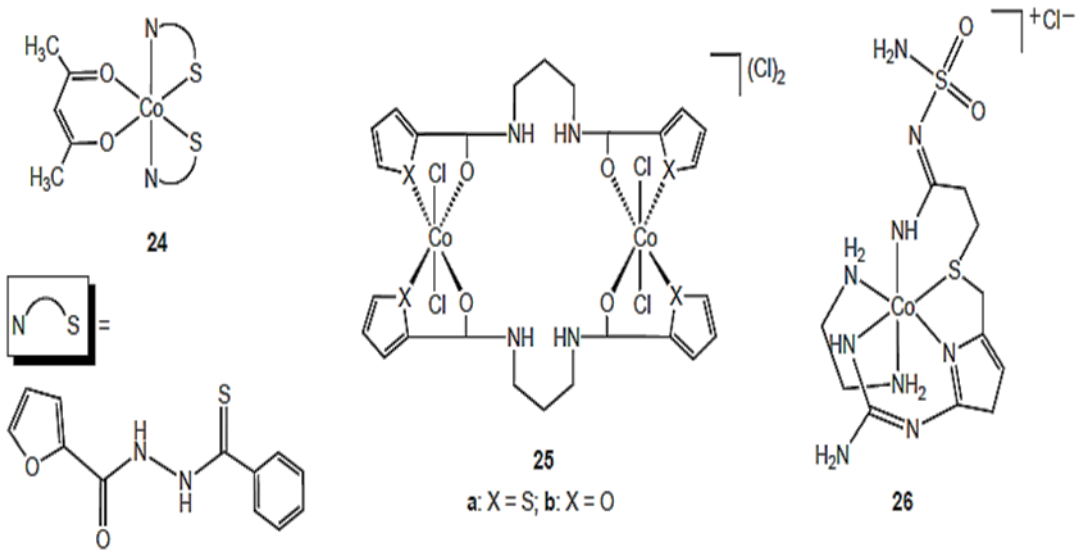
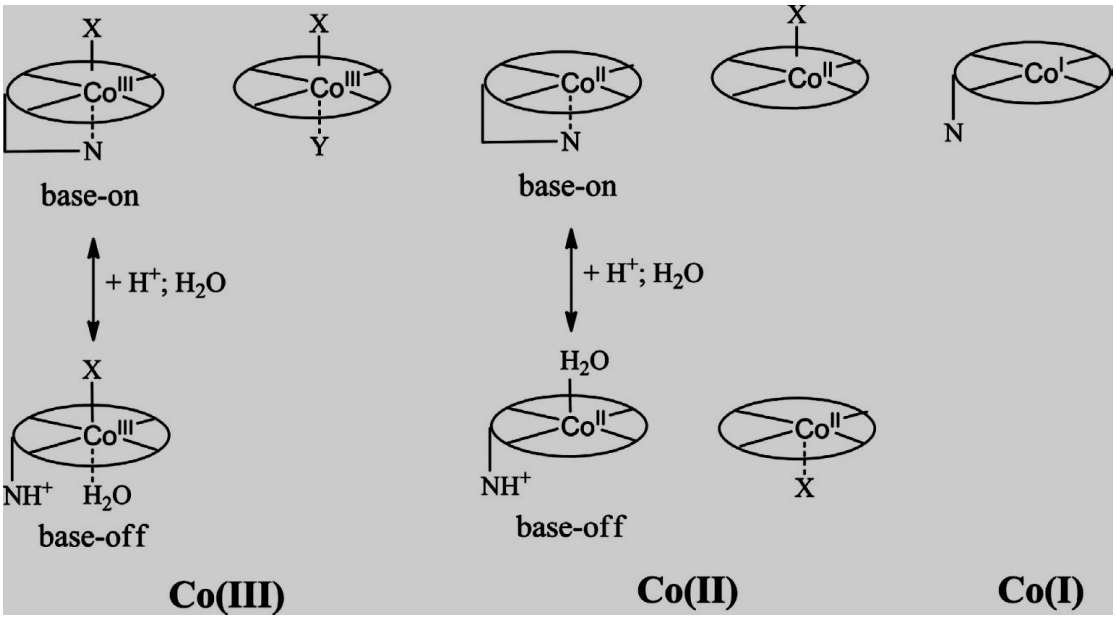


Figure (1.1): Cobalt(III) complexes containing bidentate and tetradentate nitrogen/sulfur donor ligands.

1.1.2.1 Co(III)-corrins:

Early structural investigations have been concerned mostly with the (diamagnetic) Co(III)-corrins. Throughout , the corrin-bound Co(III) center (a d^6 ion) is found to carry two axial ligands (pseudooctahedralhexacoordination);the existence of (diamagnetic) five-coordinate alkyl-Co(III)-corrins lacks convincing experimental support.

It is believed that Co(III) corrinoids are mostly hexacoordinated,while the Co(II) form tends to be pentacoordinated, and Co(I) –tetracoordinated (Kräutler,2005).



Figure(1.2) : Coordination states of Co(III), Co(II) and Co(I)-corrins.

1.1.2.2 Complexes of cobalt (III) with macrocyclic:

Ring size is the structural parameter most distinctly characteristic of macrocyclic ligands and the present work reports on the kinetics of equation and isomerization in acidic media of the cobalt(III) complexes with a series of un substituted macrocyclic ligands of varying ring sizes

(Hung ,*et al*.,1977).

1.1.2.3 Complexes of cobalt(III) with Amino acids:

It is well known that the coordination compounds of trivalent cobalt with amino acids of the type Co(am), (where am = anion of an amino acid) can exist in two geometrical isomeric forms ;.e., a violet α form and a red β form, having the peripheral and facial configurations, respectively' These compounds have been prepared by the following methods:

(1) Dissolving cobalt(III) hydroxide in the solution of the corresponding amino acid gives a production which the peripheral isomer predominates. In this way the complexes with glycine, D-alanine, L- alanine , DL-valine , L-leucine, and DL-phenyl alanine were prepared .

(2) The reaction of alkali salts of amino acids with hexaammine cobalt(III) chloride favor formation of the facial isomer of the corresponding tris (aminoacido)- cobalt(III) complex. The method was applied to the complexes of glycine, m-alanine, and L- leucine

(3) Using the reaction between alkali tricarbonato- cobaltates(III) and amino acids-glycine, DL-alanine and L-leucine in the presence of acetic acid, Mori and co-workers obtained both isomers in approximately equal amounts(Muraji Shibata,*etal*.,1967).

1.1.3 Antibacterial Activity of Cobalt(III) Complexes :

The simple Co^{3+} ion is unstable in water, but can be stabilized against reduction to Co^{2+} by coordination to ligands.

By far the most common ligand type used to stabilize the cobalt(III) ion in aqueous solution is the chelating N,O donor ligand.

Surprisingly, cobalt(III) complexes derived from this ligand donor set have found application as antibacterial or antiviral agents.

(Takeuchi,*et al*.,1999) .

1.1.4 Biological role of Co^{3+} in biological system:

Amongst various transition metals cobalt in small amounts is essential to many living organisms, including humans.

In human Co is not easily absorbed from the digestive tract. It is stored in blood plasma, as well as in the liver, kidneys, spleen and pancreas.

Cobalt, being essential metal, influences different physiological and enzymatic functions. As cobalt does not accumulate in the body, that is why Co-compounds have relatively low toxicity.

It also helps immune system ward off infections and can stimulate red blood cell production. Cobalt is needed to a mammalian body system at 0.5 mg/day above this level it creates cellular toxicity (Paternain,*etal*.,1988).

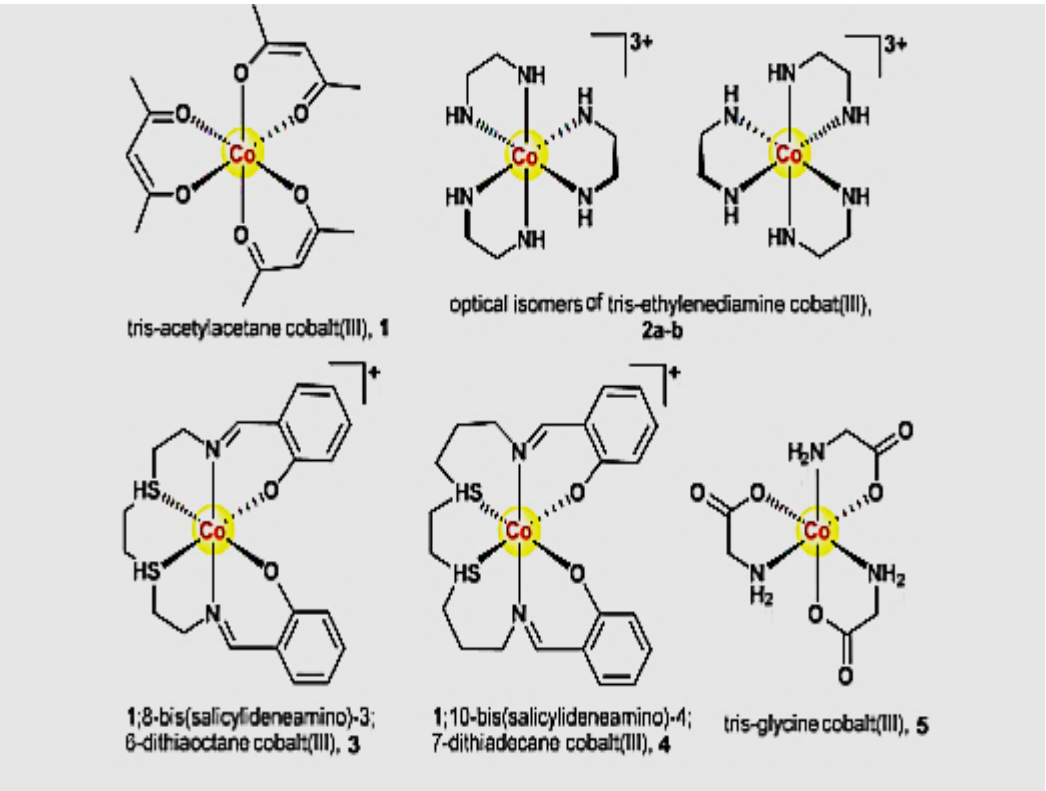


Figure (1.3): Chemical structures of cobalt(III) coordination complexes used in early biological studies.

Table (1.1): known Cobalt-containing proteins

Protien	Source	Cofactor Content	Role of Cobalt
Methionine	Animals ,yeast ,bacteria	2Co per Subunit	Hydrolysis
Prolidase	Archae	1↔2Co per Subunit	Hydrolysis
Bromoperoxidase	Bacteria	<0.35 Co per 2 Subunit	Bromination]

Apart from the importance of cobalt for functional activity of vitamin B12.

Vitamin B12 contains cobalt in a substituted corrinmacrocycle (a porphyrin relative) .

The Co^{3+} ion in vitamin B12 is stabilized by a chelating tetradentatemacrocycle known as a corrin in which the four nitrogen atoms are located in equatorial positions in the octahedral geometry (Angerer and Heinrich ,1988).

Literature review

1.2.1 Vitamin B12*(*cobalamin*):*

Cobalamins (cbls) are the most common in vivo Cobalt compounds , as well as the most complex vitamins in their structure in Nature (Gruber, et al , 2011), it is a water-soluble vitamin, which play roles in red blood cell formation, nerve cell maintenance, and methyl donation in DNA synthesis, important for human and animal metabolism (Cheng ,*et al*., 2014).

cobalamin is a member of the corrinoid series of cobalt-containing compounds and is distinguished from some other members of this group by possessing a nucleotide side chain terminating in dimethylbenzimidazole.

Vitamin B12 was discovered some 55 years ago as the (extrinsic) anti pernicious anemia factor (Hannibal, *et al*., 2007) .

The vitamin is stable in aqueous solution at room temperature. It may be heated to 120°C with little loss of activity.

Crystals of cyanocobalamin are dark red, needle-like, and contain about 12% water.

The cobalt complex was structurally characterized by X-ray analysis in the laboratory of D.C. Hodgkin .

In these studies, the unique build-up of the corrin ligand and the intramolecularly coordinating ‘pseudo-nucleotide’ function of the vitamin were discovered.

The unusual nature of the red cobalt complex induced considerable activity in the field of coordination chemistry: vitamin B12 derivatives were found to represent unique examples of kinetically labile Co^{III} complexes (Fedosov,*et al*., 2002).

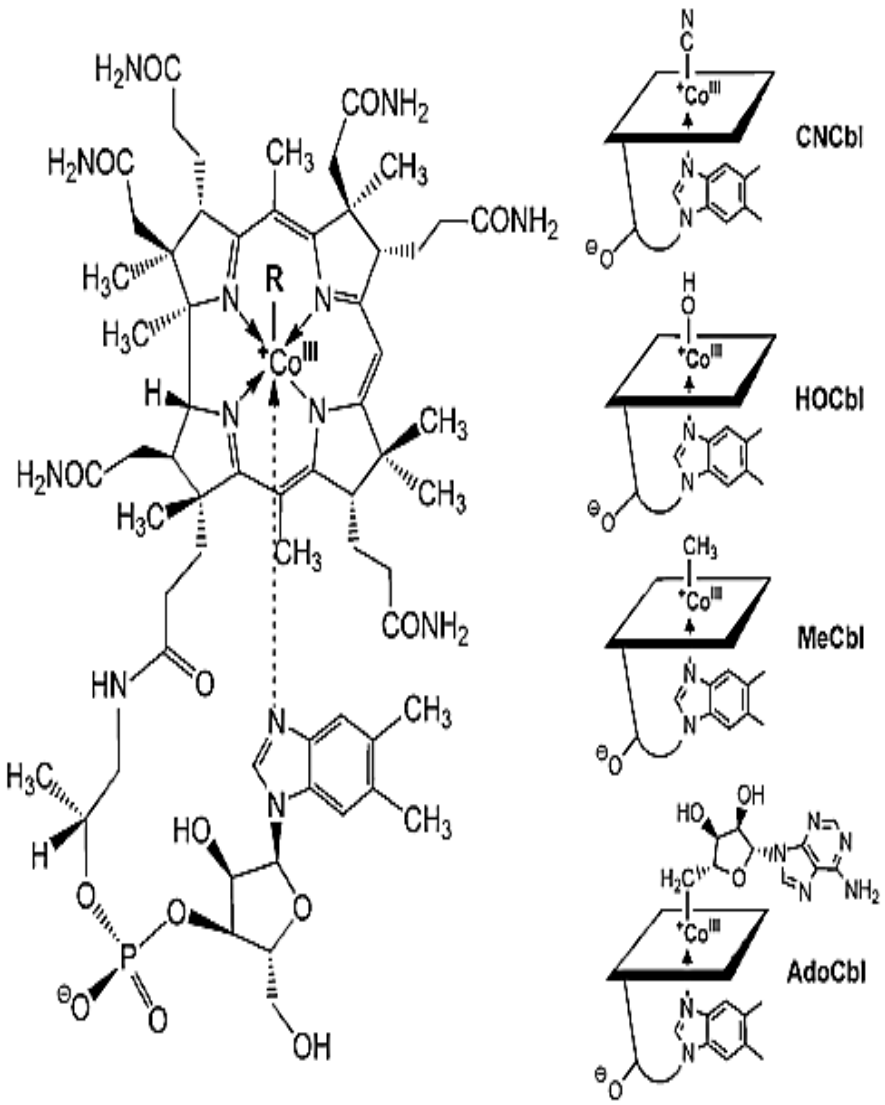


Figure (1.4): Generalized structural formula and corresponding symbols of vitamin B12 (R=CN:cyanocobalamin, CNCbl) and of the cob(III)alamins (Cbls) methylcobalamin(R=CH3, MeCbl); coenzyme B12 (R=5'-deoxy-5'-adenosyl, adenosylcobalamin, AdoCbl)and hydroxocobalamin (R=OH, HOCbl).

1.2.2 Physical properties of vitamin B12:

- B12 is obtained as a dark red crystals .
- Vitamin B12 on being exposed to air may absorbs `12 of water.
- The hydrate dark red crystals are fairly stable in air.
- It darkness at 210-220C.
- Vitamin B12is both odourless and tasteless.

- 1 g of vitamin B12 dissolved in 80 ml water .
- Its aqueous are found to be neutral in nature.
- vitamin B12 is insoluble in aceton and ether chloroform .

(Broderick and Boss, 2017) .

1.2.3 Absorption, Transport and Cellular Uptake of B12:

The vitamin B12 synthesised in microorganisms enters the human food chain through incorporation into food of animal origin.

In many animals gastrointestinal fermentation supports the growth of these vitamin B12–synthesising microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues (Watanabe , 2007).

Cobalamins cannot be synthesized by higher organisms and must be supplied with the diet. In humans, the lack of dietary Cbl or malfunctioning of absorption or of the enzymatic catalysis may provoke neurological disorders, in addition to pernicious anemia (Woodward ,*et al*., 1999) .

Mammals have developed a complex pathway, sketched on the left side of(Figure 1.5)bellow, for absorption , transportation and cellular uptake of cobalamin. This pathway involves three separate binding proteins, haptocorrin (HC), intrinsic factor (IF) and transcobalamin (TC), which form tight complexes with Cbl (Alpers and Russel , 1999).

The absorption of vitamin B12 in humans is complex,vitamin B12 in food is bound to proteins and is released from the proteins by the action of a high concentration of hydrochloric acid present in the stomach,this process results in the free form of the vitamin, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands. These glycoproteins, called R-binders (or haptocorrins), protect vitamin B12 from chemical denaturation in the stomach (Andersen,*et al*., 2010) .

The stomach’s parietal cells, which secrete hydrochloric acid, also secrete a glycoprotein called intrinsic factor.

When the contents of the stomach enter the duodenum, the R-binders become partly digested by the pancreatic proteases, which causes them to release their vitamin B12.

Because the pH in the duodenum is more neutral than that in the stomach, the intrinsic factor has a high binding affinity to vitamin B12, and it quickly binds the vitamin as it is released from the R-binders.

The vitamin B12–intrinsic factor complex then proceeds to the lower end of the small intestine, where it is absorbed by phagocytosis by specific receptors (Weir,*etal*.,1999) .

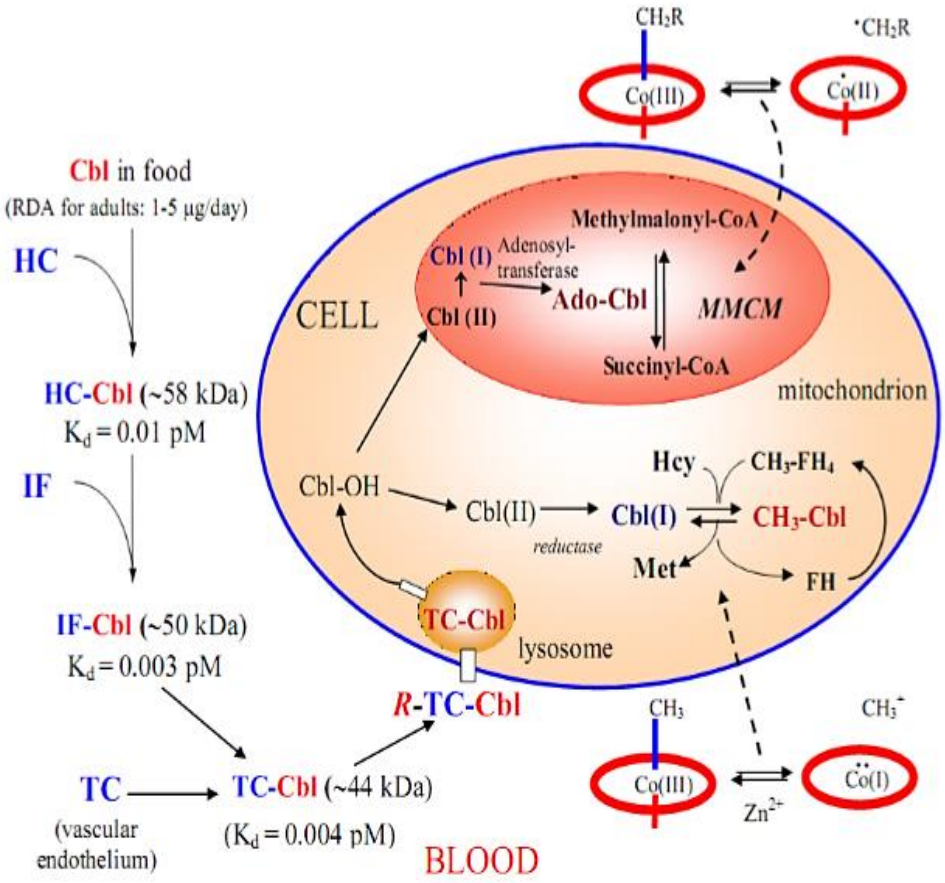


Figure (1.5) : Absorption, transport and cellular uptake of cobalamins in mammals.

1.2.4 Function of Vitamin B12:

-Is important for the normal functioning of the brain and nervous system and for the formation of blood.

-It is involved in the metabolism of every cell of the body, especially affecting DNA synthesis and regulation but also fatty acid synthesis and energy production. (Leal,*et al*., 2003).

-Vitamin B12 (cobalamin) derived cofactors are used for two important reactions in humans **:**

(1) Methylmalonyl CoA mutase requires 5-deoxyadenosylcobalamin (known as adenosylcobalamin) involved in fat metabolism.

(2) Methionine synthase (cofactor methylcobalamin) catalyses the conversion of homocysteine to methionine ,important in production of red blood cells.

Therefore, Vitamin B12 controls:

i. normal nerve cell activity – formation of myelin basic protein
ii. DNA and RNA replication .
iii. production of the mood-affecting substance SAM (S-adenosyl-L-methionine).
iv. Vitamin B12 works closely together with vitamin B9 (folate) to regulate the formation of red blood cells and to assist in the function of iron.

(Green ,*et al*., 2017).

v. Vitamin B12 is important for the activity of enzymes within cells that control fat, amino acid and carbohydrate metabolism .
vi. Cyanocobalamin promotes normal growth and development; treats pernicious anemia .
vii. Vitamin B12 helps in the maintenance of the central nervous system .
viii. B12 plays a vital role in the metabolism of fatty acids essential for the maintenance of myelin. Nerves are surrounded by an insulating fatty sheath comprised of a complex protein called myelin.

Deficiency of vitamin B_{12} affects immunologic and hematologic parameter in the body.

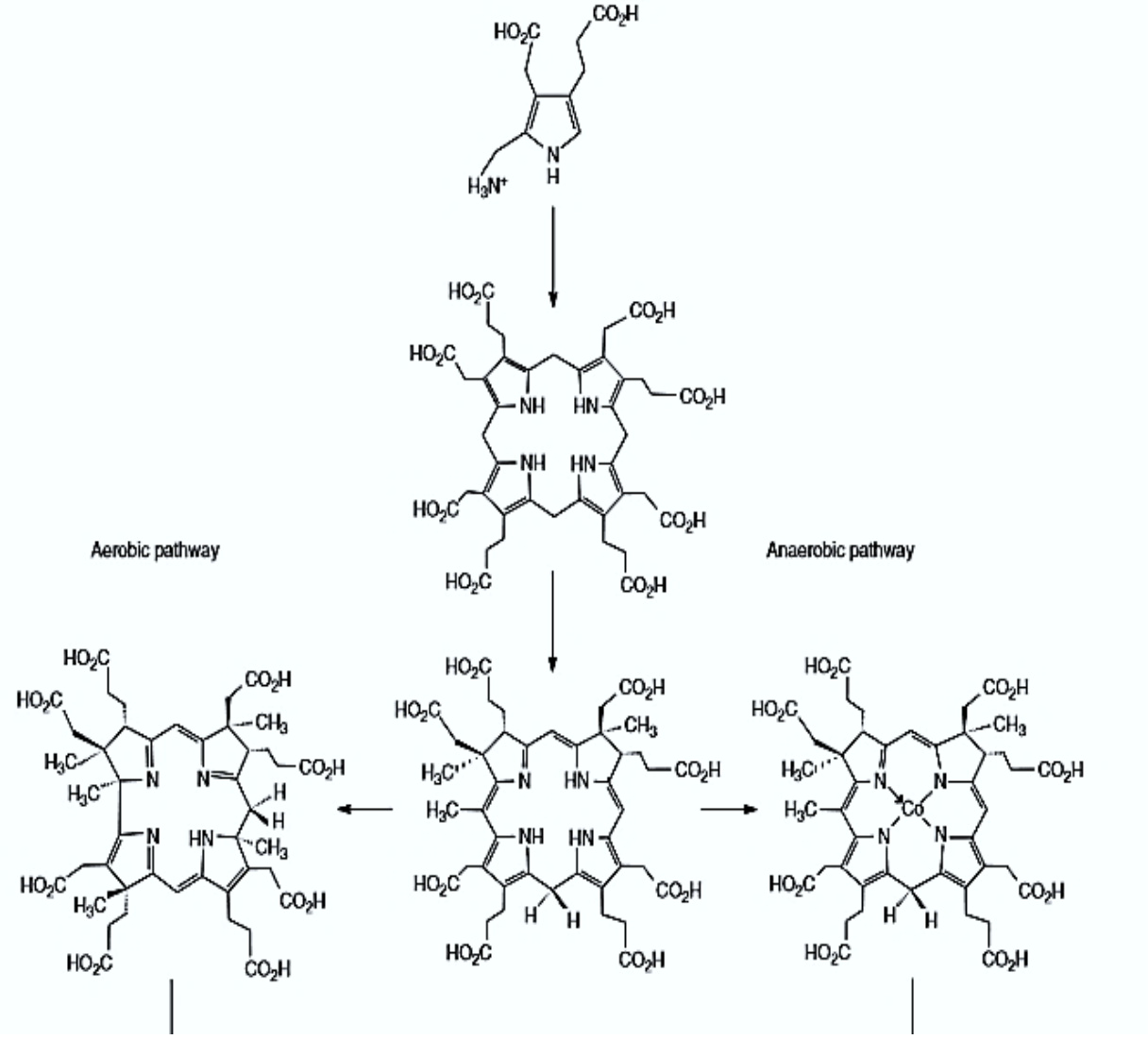
1.2.5 the stability of vitamin B12:

Cyanocobalamin is the most stable form of vitamin B12 and is normally used for fortification.

Crystalline forms are stable when protected from light, strong light or the presence of oxidising agents will however destroy the vitamin(Pratt, 1999) .

1.2.6 Synthesis of vitamin B12:

The preparation of corrins and the total synthesis of vitamin B12 was achieved in the 1970s in the laboratories of Eschenmoser and Woodward (Eschenmoser and Wintner , 1977).



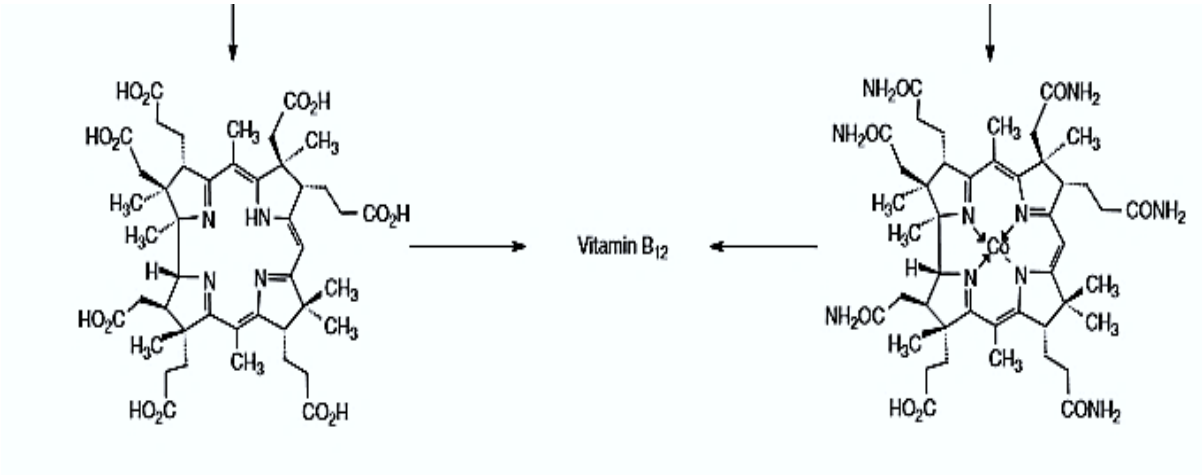


Figure (1.6) : Outline and some central intermediates in the two main known pathways of the biosynthesis of vitamin B12, the 'aerobic path' (left) and the 'anaerobic path' (right).

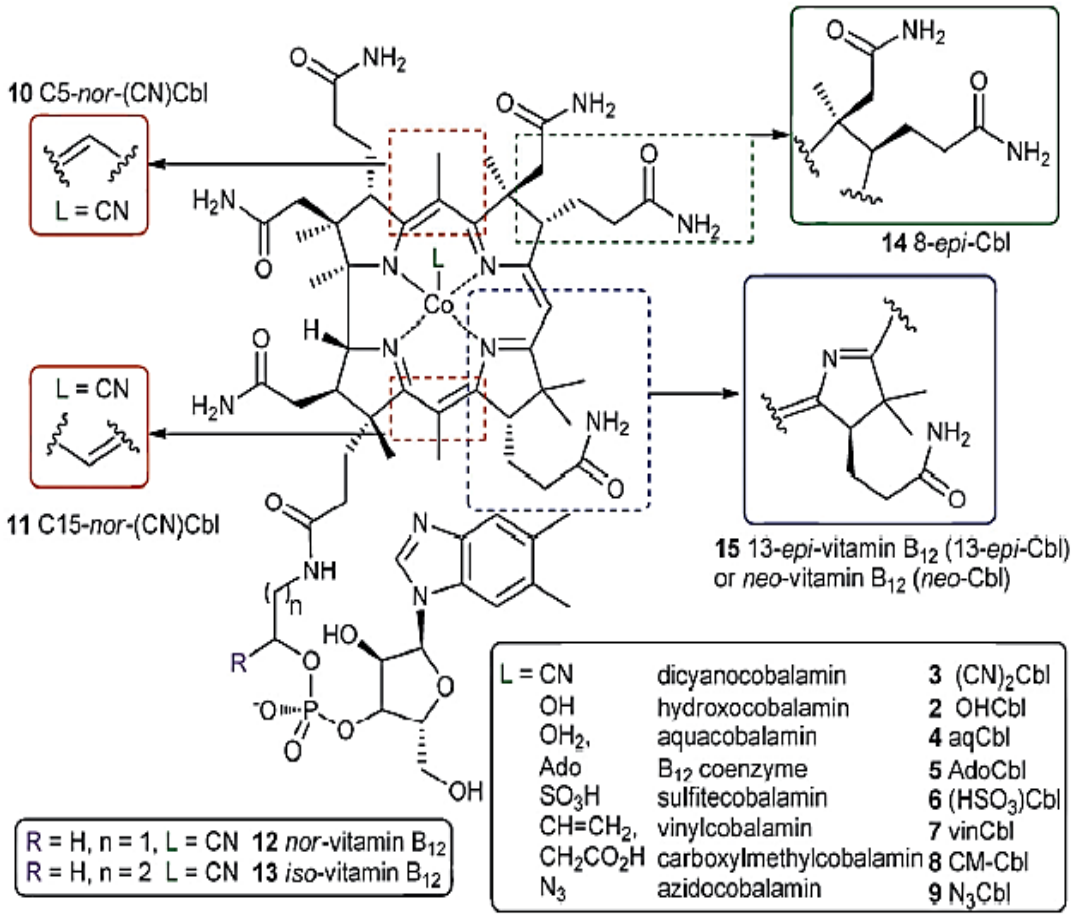


Figure (1.7) : Nomenclature of cobinamide and its derivatives.

1.2.8 Aquacobalamin(vitamin B12 a) :

Cobalamins, and especially aquacobalamin (H_2OCbl), are capable of interacting with such ligands as thiols , H_2S, CN^-, pyridine, cyanamide , SCN^-, N_3^-, and $S_2O_3^{2-}$.

Many of these interactions find practical application .For example, the high reactivity of cyanide in relation to aquacobalamin and the low toxicity of the resulting complex are responsible for the high efficacy of H_2OCbl as an anti-dote against CN^- (Thompson ,*et al*.,2012) .

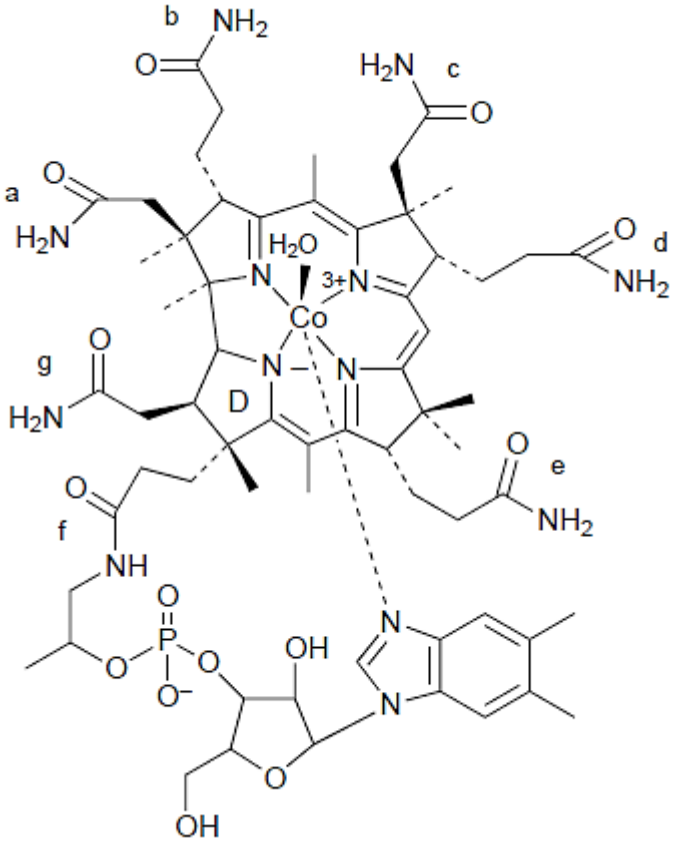


Figure (1.8): Aquacobalamin (vitamin B12a), in which Co(III)is co-ordinated in the equatorial plane by corrin. The axial ligand on the lower (α) face is 5,6-dimethylbenzimidazole (dmbzim), and that on the upper (β) face is H_2O

1.2.9 Chemistry of vitamin B12:

The various forms of the vitamin have either a cyanide, hydroxo, deox-yadenosyl, or a methyl group attached to the cobalt atom.

When exposed to light, cyanocobalamin ,deoxyadenosylcobalamin, and methylcobalamin are converted to hydroxocobalamin or aquocobalamin by photolysis (Demerre and Wislon ,1956) .

Hydrox- ocobalamin has an OH- group and aquocobalamin an HO group bound to the cobalt atom. Prolonged exposure to sunlight results in the conversion of 10% of cyanocobalamin to hydroxocobalamin for each 30 minutes of exposure , this change can be reversed in the dark and there is no loss of vitamin activity.

Because of this light sensitivity the biologically active form of the vitamin, that is, the coenzyme form deoxyadenosylcobalamin, was not recognized until 1958 (Barker,*et al*.,1958) .

1.2.9.1 Oxidation States of cobalt ion in vitmin B12:

Cyanocobalamin or any of the alkylcobalamins contain cobalt formally in the (3+) oxidation state .

As with all cobalt(III) complexes these cobalamins are diamagnetic (aside from a certain small amount of temperature-independent paramagnetism).

The cobalt (III) species, usually as cyano or aquocobalamin, can be reduced in one-electron steps to a cobalt(II) species B12r, and then to the cobalt (I) species B12 S.

This reduction can be accomplished by several reducing agents.

The most common methods of carrying out the reduction to the B12 a level include reduction by zinc dust in ammonium chloride solution, sodium borohydride,chromous ion (pH 9-10), or by electrochemical means(Dereven ,et al.,2016) .

(B12r) a brown cobalt (II) species, is a low-spin 8 complex. As such, it contains one unpaired electron and is the only paramagnetic B12 derivative, the unpaired electron resides in the $3d_{Z2}$ orbital. Under most conditions (B12 r) is a 5-coordinate complex .

The compound can be quite easily oxidized back to aquocobalamin by atmospheric oxygen and therefore must be stored anaerobically. It has been reported that B12 r in solution is able to disproportionate to (B12 s) and (B12r) (Yamada,*et al*., 1968) .

1.2.9.2 Kinetic of cobalamin:

The cobalt complex of the B12 derivatives is electrochemically active.

Each of the three possible formal oxidation states of cobalt in cobalamins has a preferred coordination environment (i.e., octahedral, square pyramidal or square planar for Co(III),(II)or(I) respectively),with each electron transfer accompanied by the gain or loss of one axial ligand (Rose ,*et al* .,1984) .

1.2.9.3Reactions on the central cobalt ion:

The central cobalt ion in (CN) Cbl (1) is coordinated to the cyanide ligand and to the nitrogen atom of dimethylbenzimid- azole. There is an equilibrium between the free (base-off) and coordinated (base-on) form which is shifted by adjusting pH (Brown,2005).

The exchange of ionic ligands is straightforward as it involves aquacobalamin formation and its subsequent treatment with an appropriate salt solution e.g. KCN, KN_3, NaSCN , etc.. (Scheme 1.4).

Reactions occurring at the cobalt ion on vitamin B12 (1) have been at the centre of research for many years due to its biological importance (Banerjee,1999).

Cobalt-alkyl vitamin B12 analogues are produced by enzymatic systems as intermediates in the synthesis of coenzymatic forms of corrinoids e.g. methylcobalamin or adenosylcobalamin, which are cofactors for such vitamin B12-dependent enzymes as methyltransferase, methylmalonyl-CoA mutase, dioldehydratase , glyceroldehydratase , deaminases, etc.

The process can be monitored visually as a colour change is observed Cbl (red) reduces to Co(II) (brown) and then to Co(I) (blue/green) (Figure 1.9).

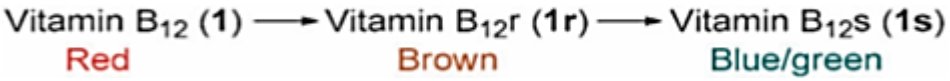


Figure (1.9): change in colour

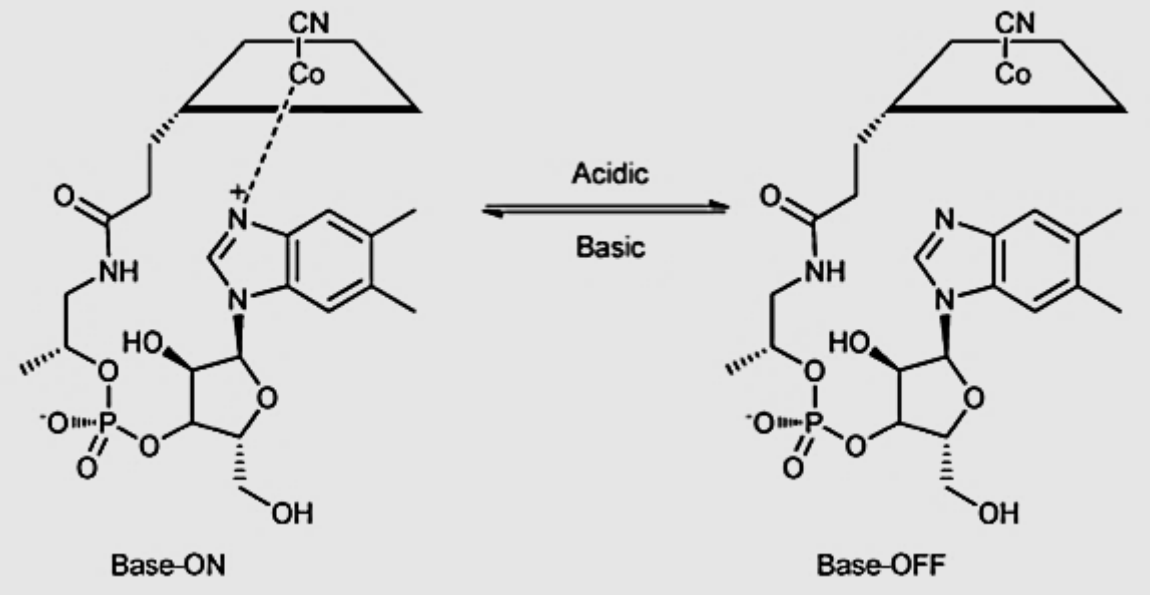


Figure (1.10) Vitamin B12 base on/base off modes.

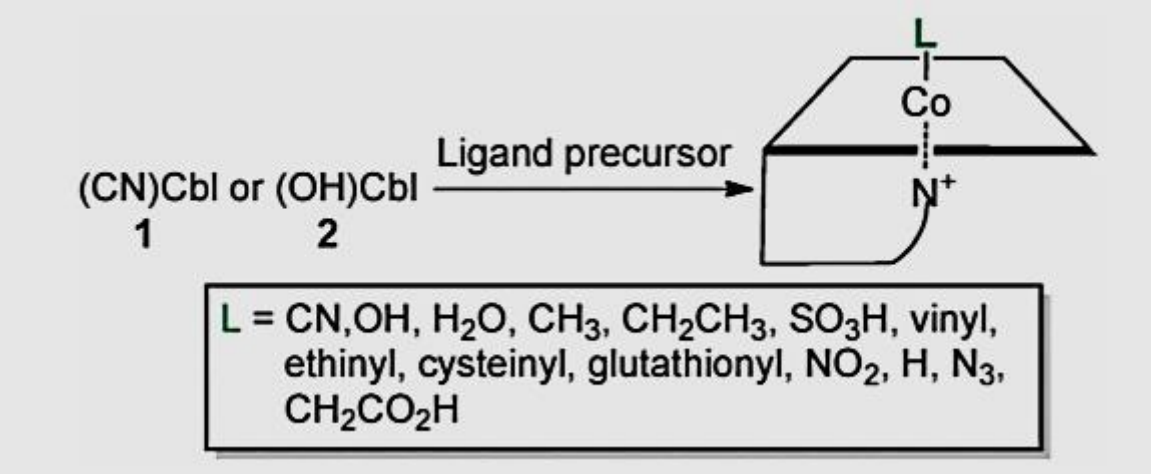


Figure (1.11) Synthesis of various Cbl analogues.

1.2.10 B12-Structure:

Analysis of vitamin B12 (CNCbl) with the help of X-ray crystal diffraction provided the first glimpse at the structure of a corrin complex, a structural and biosynthetic relative of the better known natural porphyrins.

In cobalamins (Cbl), the Co atom is equatorially coordinated by the corrin ligand possessing seven amide side chains (a-g),the numbering scheme

for the atoms of this complex introduced by Dorothy Hodgkin is shown in Figure (1.13b).

The corrin contains four reduced pyrrole rings, A-D, with a direct connection between rings A and D, and has a single helical sense of absolute R-configuration of its chiral centres at C1 and C19. Chain (f) is connected through an amide bond to a nucleotide, whose the 5,6-dimethylbenzimidazole base coordinates Co at the axial position on the α side (lower side) of the corrinmacrocycle(Koutmos ,*et al*.,2008).

Cobalamins with the Co in the oxidation state +3, are generally octahedral, with the axial X ligand coordinated on the β side (upper side) of the corrinligand in the base-on form (Figure 1.13c, left side). Cobalamins assume the base-off form (Figure 1.13c, right side) upon protonation of the coordinated NB3 atom and benzimidazole is substituted by an exogenous ligand. In the Co(II) oxidation state, the cobalamin has no β axial ligand and it is generally known as cob(II)alamin or B12r, whereas in the Co(I) state it is presumably tetracoordinate in a base- off form and without the β axial ligand (cob(I)alamin or B12s). Corrinoids having structural modification with respect to cobalamins, such as a different base in the nucleotide loop or with changes in the corrin ligand, are preferentially indicated as cobamides (Cba), specifying the axial ligand (s) and the nucleotide base, if necessary. (Kräutler,2005)

The cobinamides (Cbi) are related nucleotide-free corrinoids, in which the X and Y ligands in the axial position are specified by α and β according to their position on the two sides of the corrin ring.

As already stressed, CNCbl is not known to have a direct biological role, whereas MeCbl.

(X=CH_3 in Figure 1a) and AdoCbl (X = 5'-deoxyadenosylcobalamin in Figure 1a) are cofactors of several enzymes.

MeCbl is the cofactor of several methyltransferases, such as methionine synthase (MetH), which catalyzes methionine biosynthesis both in mammals and bacteria. Methylcorrinoids, including MeCbl, are also cofactors of enzymes participating in the carbon dioxide fixing pathway in anaerobic bacteria (Sauer and Thauer ,1999) .

The enzymatic mechanisms for the methyl transfer require the formally heterolytic cleavage and formation of the Co-Me bond often in presence of an additional cofactor, either a Zn^{2+} ion or a [Fe_4S_4] cluster (Koutmos,*et al*.,2008).

AdoCbl is the cofactor of several enzymes, eliminases and isomerases (or mutases), which catalyze the 1,2 shift of an H atom and an electronegative group on adjacent carbon atoms.

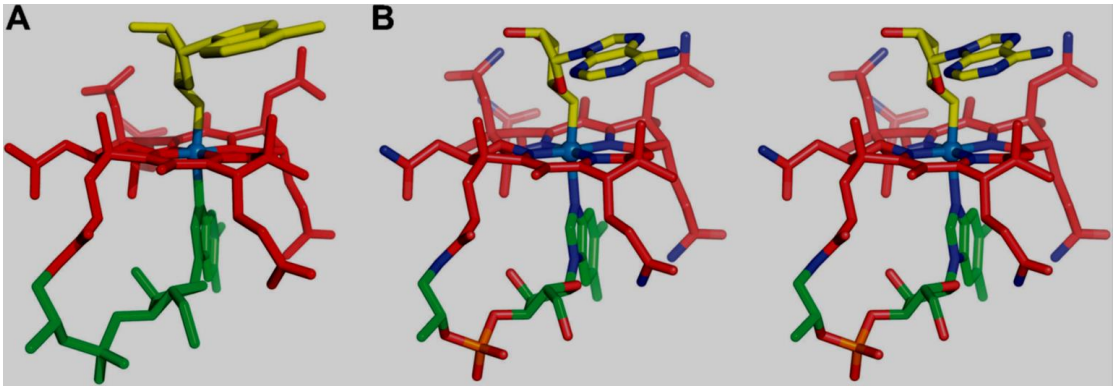


Figure (1.12) : Three dimensional model of coenzyme B12 (AdoCbl) from X-ray crystallography.12 (A) structure of AdoCbl in stick representation; colour coding: corrin moiety (red), the nucleotide loop (green), the cobalt-ion (blue) and the organometallic adenosyl group (yellow); (B) stereo-view of AdoCbl (hetero-atoms: N = dark blue, O = red, P = yellow, Co = light blue), etc

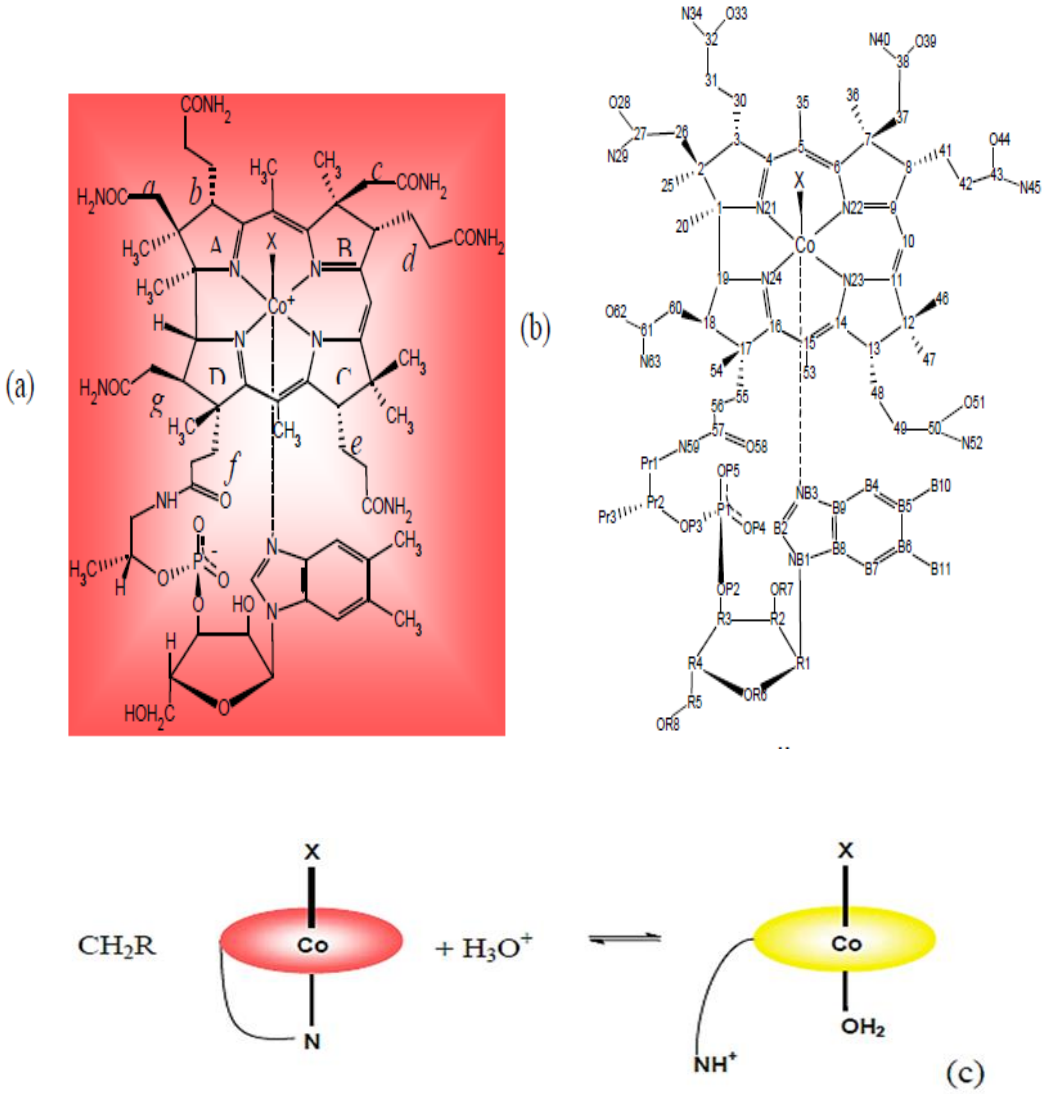


Figure (1.13) : (a) Structural formulas of vitamin B_{12} (X=CN) and of the biologically active cobalamins (X=Ado, X=CH_3); (b) conventional atoms nomenclature for cobalamins; (c) base-on form (left side) and base-off form (right side); only the number or the symbol are indicated for the carbon atoms.

The internal moiety of the corrin ligand has a π delocalized system involving the N and the sp^2 C atoms (Figure 1.12). The four main resonance structures of the corrin moiety are shown in Figure (1.13 a). Accurate experimental values of the distances of the equatorial moiety indicated that they are scarcely affected by the kind of the X axial ligand. Furthermore, the Co-N distances involved in the five- membered ring are significantly shorter than the other two equatorial Co-N distances (by about 0.02 Å).

The experimental C-C and C-N bond lengths within the delocalized moiety have a trend which reflects the approximately two-fold symmetry with respect to the axis passing through Co and C10 (Figure 1.13b) (Randaccio, *et al*.,2009) .

This trend can be fairly well interpreted on the basis of the four main resonance structures of Figure 2a. In fact, linear relationships can be found between the experimental values of the C-C and C-N distances and the corresponding bond orders, roughly derived from the four resonance structures. The trend of the C-C single bond lengths of the peripheral moiety of the corrin which vary from 1.508 Å of the C8-C9 bond to 1.580 Å of the C1-C2 bond, reflects the different hybridization and the number of non-H substituents at the bonded C atoms Figure(1.13b).

The preferred mode of deformation of the corrin ligand is represented by a folding towards the X axial ligand, about an axis approximately bisecting the C1-C19 bond and passing through C10.

The deformation is measured by the folding angle φ, which is generally calculated as the dihedral angle between the planes Figure (1.13 c) passing through N21, C4, C5, C6, N22, C9, C10 and C10, C11, N23, C14, C15, C16, N24 Figure(1.13 c). The folding angle φ roughly decreases with increasing Co–NB3 distance, i.e. with an increase in the trans influencing ability of X, rather than with an increase of its bulk.

In fact, the steric pressure of the benzimidazole residue (indicated by the double blue line in Figure (1.13 c) on the equatorial ligand is released when the Co-NB3 bond lengthens because of the increase in the trans influencing ability of X (Randaccio ,*et al*.,2006).

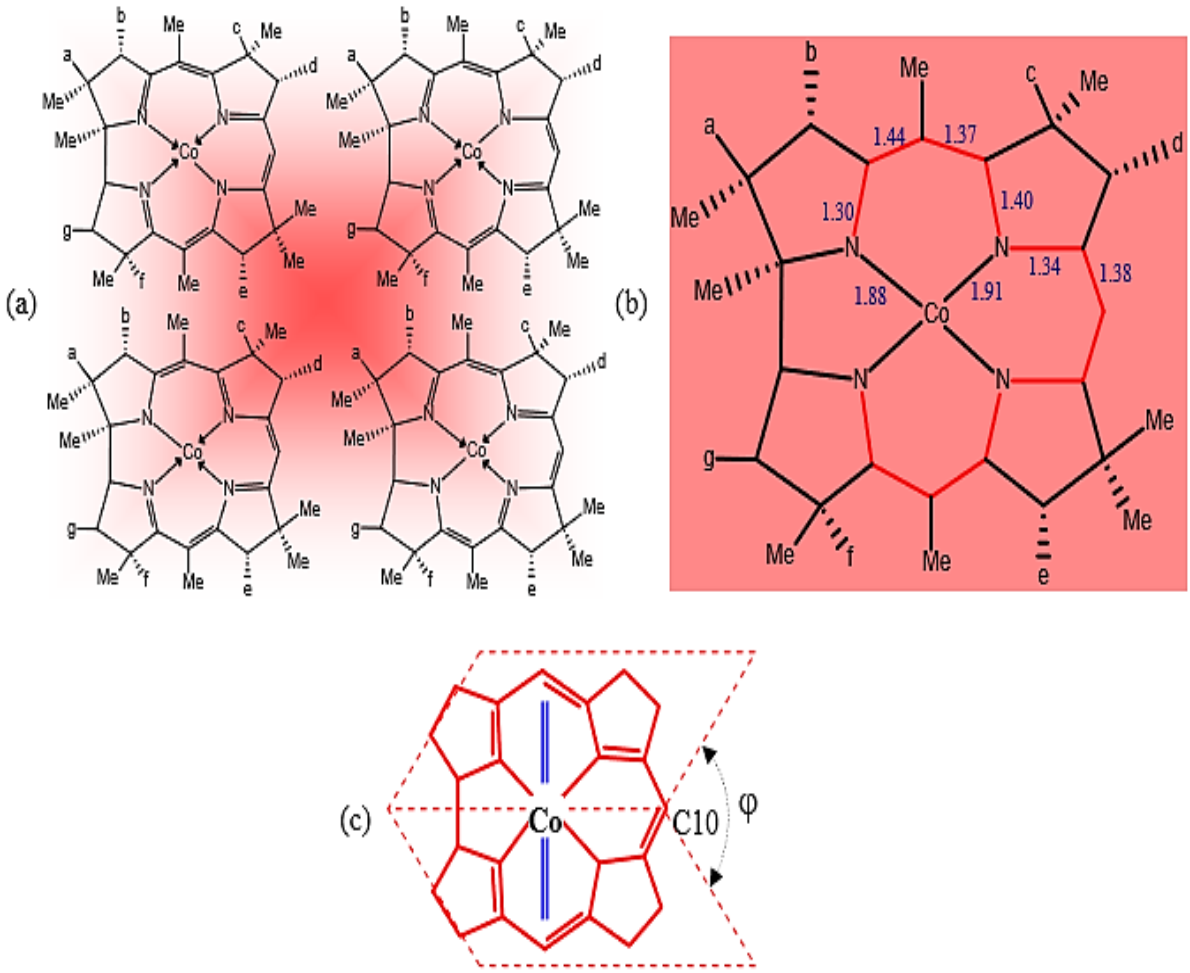


Figure (1.14) : a) The main four resonance structures for the delocalized corrin system; b) mean bond lengths within the delocalised moiety of the corrin nucleus; c) the folding angle . The double blue line represents the trace of the 5,6-benzimidazole group.

For several XCbl complexes, with X varying from H_2O to NO, the Co-NB3 axial distance lengthens by about 0.4 Å, being 1.925Å in the aquocobalamin (H_2OCbl+) and 2.349 Å in NOCbl , respectively.

The lengthening of that bond trans to X reflects the increase in the electron σ-donating ability of X (electronic trans-influence).

The alkyl groups are among the stronger *trans*-influencing ligands as compared to the "inorganic" ligand, the order of increasing trans-influencing ability of X donor atom being O < N < S < C. For the same X group, the lengthening of the Co-NB3 bond was found to be more enhanced with respect to cobaloximes and $XCo(NH_3)_5$ (Randaccio,*et al.*,2009).

On the contrary, the Co-X distances do not vary when compared to the analogous ones in cobaloximes or in $XCo(NH_3)_5$, except when X is either a weak electron σ-donating group, such as H_2O, or a good electron π-acceptor, such as CN^-. This was interpreted as a consequence of the electronic cis-influence of the equatorial moiety on the axial bonds.

In alkylcobalamin the increase in bulk of the alkyl group, R, provokes a weakening of the Co-C bond due to the steric interaction of R with the corrin ligand (*cis*-influence). Interestingly, the X-ray structure of NOCbl has shown that the inorganic nitroxyl ligand (with a bent Co-N-O geometry) exerts a significantly larger trans-influence, as measured by the length of the Co-NB3 bond, than that of an alkyl group.(Hannibal , 2009).

However, NMR and UV-Vis spectroscopies have furnished evidence that a significant fraction, about one third, of NOCbl in solution is present in base-off form.

In alkylcobalamins (including CNCbl), it has been found that both Co-NB3 and Co-C distances increase when both the bulk and the electron donating ability of X increase: this trend is called inverse trans-influence, in contrast to the regular trans-influence which occurs when the Co-X shortens and the trans bond lengthens, as is observed in a series of cobalamins with X = ligands containing a $S(sp^3)$ donor (Kuta ,2009) .

The coordinated water molecule in H_2OCbl (vitamin $B_{12}a$) is easily displaced by several other Z ligands according to the reaction in aqueous solution:

$$\mathbf{H_2OCbl + Z \leftrightarrow ZCbl + H_2O}$$

The equilibrium constant, K, for the above reaction varies by several orders of magnitude when the Z ligand is varied.

For example, the trend of log K for the following Z ligand (K in parentheses) is: CN^-(14.1) >> $SO3^{2-}$ (7.8) > OH^- (6.2) >N^{3-} (4.9) > I^- (1.5) >Cl^- (0.1).

There is evidence that this order of the binding affinity of the Z ligands to cobalt, as expressed by the K values, is also maintained, at least qualitatively, in the cobalamins bound to protein (Kräutler,2019).

In addition to the reactivity of Co at the axial position, the elevated number of functionalities available on the side chains of the corrin allows many reactions,which give a huge number of derivatives, including bioconjugates(Kräutler,2019) .

1.2.11 B12 Conjugate:

In principle, conjugation may occurs by:

i) reaction with the carboxylic groups prepared by hydrolysis of the peripheral corrin amide side chains.
ii) by esterification at cobalamin'sribosyl 2'-OH and 5'-OH (OR7 and OR8, respectively
iii) by reductive alkylation at the β axial position of the Co(III) centre.
iv) by coordination to the N atom of the β axial CN group in CNCbl(Mathews, *et al*.,2007).

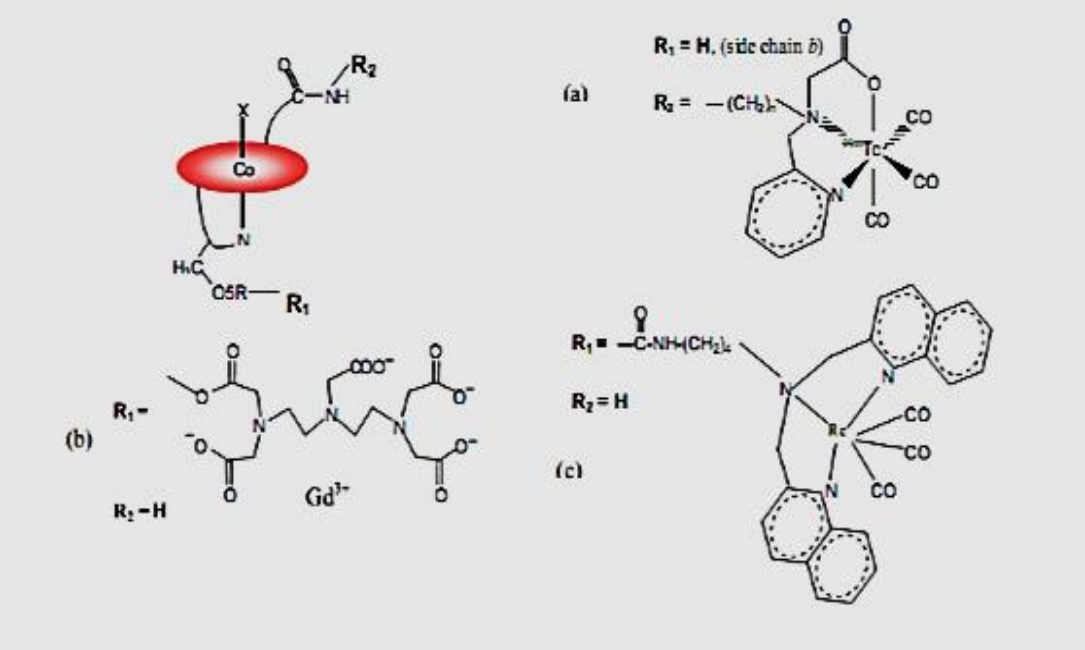


Figure (1.15) : Some more recent B12 bio conjugates.

Very recently, two bio conjugates, namely CNCbl-DTPA-Gd (DTPA= diethylenetriamine- N,N,N',N",N"'-pentaacetic acid) (Figure 15b) and the homologous CNCbl-TTHA-Gd (TTHA = triethylenetetramine-N,N,N',N",N"',N"'-hexaacetic acid)(Siega,*et al*.,2009).

B_{12} conjugates have opened further possibilities to find B_{12} conjugates for cancer diagnostics and treatment(Viola,*et al*.,2009).

1.2.12 B12–protein interactions

Three major classes of enzymes are known that use B12-molecules as their ‘small’ cofactor ligands: corrinoiddehalogenases, methyltransferases and the coenzyme B12-utilizing enzymes.

(Brown,2005).

Several mutases, dehydratases ,a deaminase and a ribonucleotidereductase belong to the latter group.

In addition, the proteins involved in B12-transport also exhibit remarkable interactions with the ‘small’ B12-molecules.

Members of all three classes of the B12-enzymes are important in microorganisms, and except for the dehalogenases in human and animal metabolism (krautler, *et al*.,1998).

1.2.13 B12 interactions with biological macromolecules :

The structure and size of B12-derivatives provide unique possibilities for their interaction with biological macromolecules.

With proteins, several topologically different binding modes for ‘complete’ corrinoids and B12-cofactors are document that appear to correlate largely with the functional role of the B12-containing assembly (Larsson,*et al*.,2010) .

In B_{12}-dependent enzymes the ‘complete’ corrinoid B12-cofactor is surrounded entirely by the protein and is bound at an interface between two subdomains(or subunits) of the enzyme.

Typically the plane of the corrin ligand is oriented nearly parallel to the protein interface so that the upper (organometallic ligand) and the lower

(nucleotide function) are exposed to different domains (subunits) of the protein (Fig 1.16).

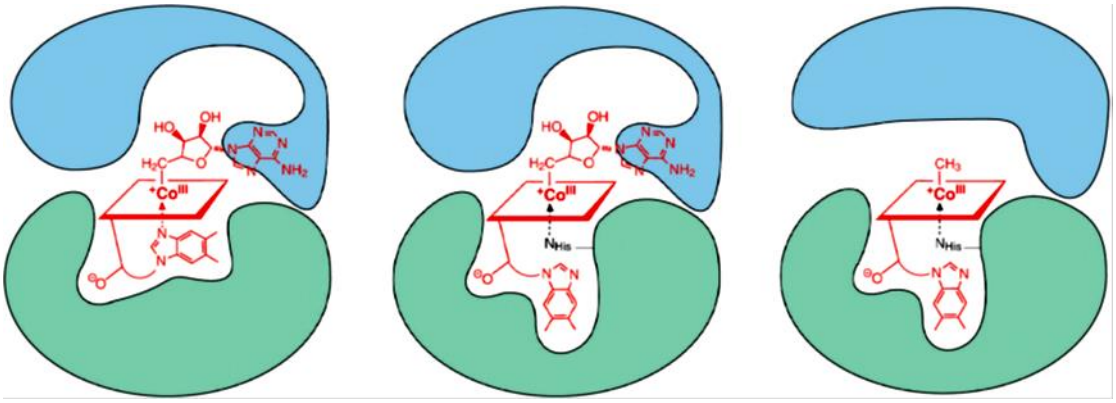


Figure (1.16) : Topological analysis of typical B12-binding in B12-dependent enzymes.

This binding mode may be crucial in providing the needed flexibility for catalysis and in helping to activate adenosylcorrinoids (like AdoCbl) in a 'mechanical' way towards formation of a deoxyadenosyl radical by (Co–C)-bond hemolysis.

Likewise it may allow for the crucial positioning of the reaction partners in methyl group transferases, where control of the methyl group transport by a fascinating multi-domain shuttling is critical.

In the latter enzymes, the B12-cofactors are bound base-off, whereas AdoCbl may be bound either base-off (as in the carbon skeleton mutases) or base-on (as in dioldehydratase and in ribonucleotidereductase) in the AdoCbl-dependent enzymes.

Binding of cobalamins to proteins of B_{12}-transport in mammals occurs both very fast (typical k_{on} ca. 10^7 to 10^8 M^{-1} S^{-1}), as well as exceptionally tight ($K_d$ca. 10^{-14} to 10^{-15} M) and highly selectively for the B12-'base-on' forms (Larsson,*et al*.,2010).

Such an efficient B12-binding is achieved by a sideways mode of binding at the interface that is provided by two domains (domain A and B) of the protein, and which results in a nearly perpendicular orientation of the plane of the corrin ring to the binding surfaces (Figure 1.16).

The B12-cargo again is completely surrounded by the protein, although this mode of binding ensures significant tolerance with respect to binding of cobalamins that differ by the nature of their 'upper' ligand (e.g. the structures of H_2OCbl, MeCbl and AdoCbl) (Fedosov,*et al*,.2002).

Binding kinetics suggest the well-structured B-domain at the C-terminal to provide the pre-structured binding interface for rapid direct recognition of the cobalt-corrin moiety of the binding substrate.

The complete binding interface is structured subsequently to provide direct and water mediated contacts with about 90% of the bound B12-molecule.

Proteins involved in transport of corrinoids in microorganisms have adapted to a variety of natural corrinoids (besides the cobalamins) and they also appear to make use of more divergent set of binding modes (Gallo,2008).

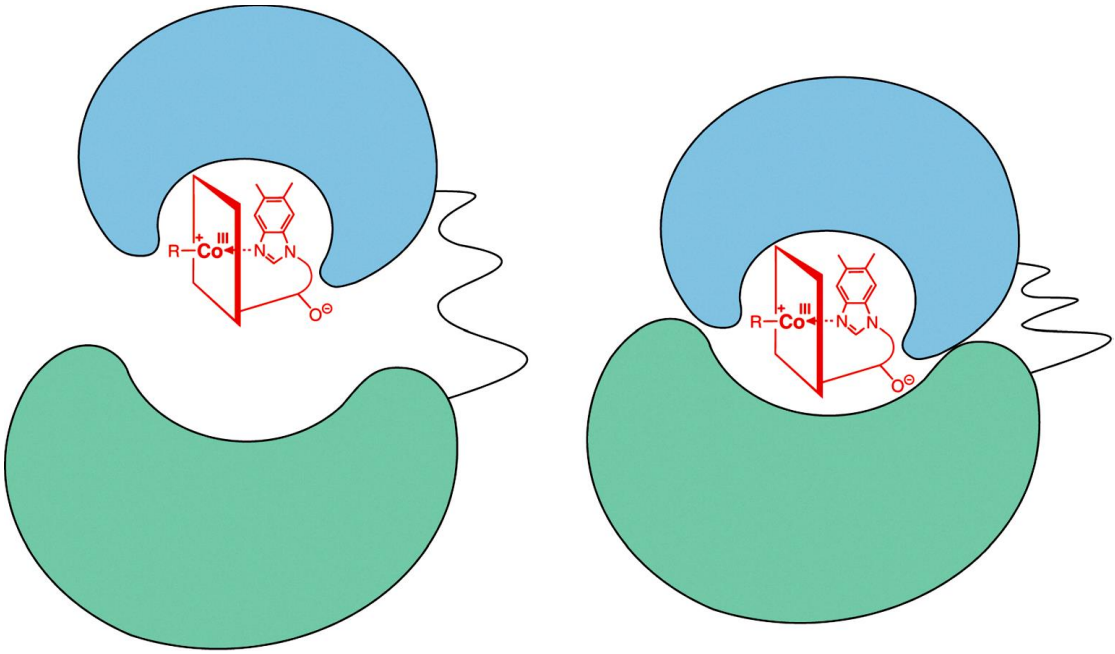


Figure (1.17) : Topological analysis of typical B12-binding in mammalian

proteins of B12-transport. Complete corrinoids are bound ‘sideways’ at the interface of two major domains.

1.2.14 Molecular Docking :

In the field of molecular modeling, molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex .

Knowledge of the preferred orientation is used to predict the strength ofassociation or binding affinity between two molecules using scoring functions.

The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates and lipids play central role in signal transduction.

Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g. agonism/ antagonism) .

Therefore docking is useful for predicting both the strength and type of signal produced.

Docking is frequently used to predict the binding orientation of drug candidates to their protein targets in order to predict the affinity and activity of the small molecule(Halperin,*et al*.,2002).

Hence docking plays an important role in the rational design of drugs .

The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand so that the free energy of the overall system is minimized.

Molecular recognition plays a key role in promoting fundamental biomolecular events such as enzyme-substrate ,drug-protein and drug-nucleic acid interactions (Davis,*et al*.,2003).

Detailed understanding of the general principles that govern the nature of the interactions(van der Waals, hydrogen bonding, electrostatic) between the ligands and their protein or nucleic acid targets may provide a framework for designing the desired potency and specificity of potential drug leads for a given therapeutic target .

Practical application of this knowledge requires structural data for the target of interest and a procedure for evaluating candidate ligands.

A variety of computational docking methods are available,these provide the ranking of potential ligands with respect to their ability to interact with given target.(Bleicher ,*et al*.,2003)

Docking of a small molecule to a biological target involves efficient sampling of possible poses of the former in the specified binding pocket of the latter in order to identify the optimal binding geometry, measured by a user-defined fitness or score function.

X-ray crystallography and NMR spectroscopy continue to be the primary source of 3D structural data for protein and nucleic acid targets.

When proteins of unknown structure have high sequence homology to known structures, homology modeling can provide a viable alternative by generating a suitable starting point for 'in silico' discovery of high affinity ligands (Desjarlais, *et al.*,1988).

Databases of drug like molecules such as MDDR or CMC , as well as other small molecule databases including ACD, CSDand NCI are available.

During computational docking, a pose is generated, scored and compared to the previous pose(s).

The current pose is then accepted or rejected on the basis of the score for that pose.

A new pose is then generated, and the search process iterates to an endpoint. Thus searching and scoring can be tightly coupled in docking.

Reliable rank ordering of the ligands based on their docked scores such that the scores correlate with experimental binding affinities appears to be even more challenging than searching the conformation and orientation space. (Tame ,1999).

A recent trend has been to employ consensus scoring (apply a number of score functions to the same docked pose identified by docking to eliminate false positives.

In silico approaches need to be robust and fast in order to have a major impact on lead identification (Desjarlais, *et al*.,1988).

1.2.14.1 Molecular Docking Approaches:

Two approaches are particularly popular within the molecular docking community.

One approach uses matching technique that describes the protein and the ligand as complementary surfaces.

The second approach simulates the docking process in which the ligand-protein pair wise interact energies are calculated (Feig, *et al*.,2004) .

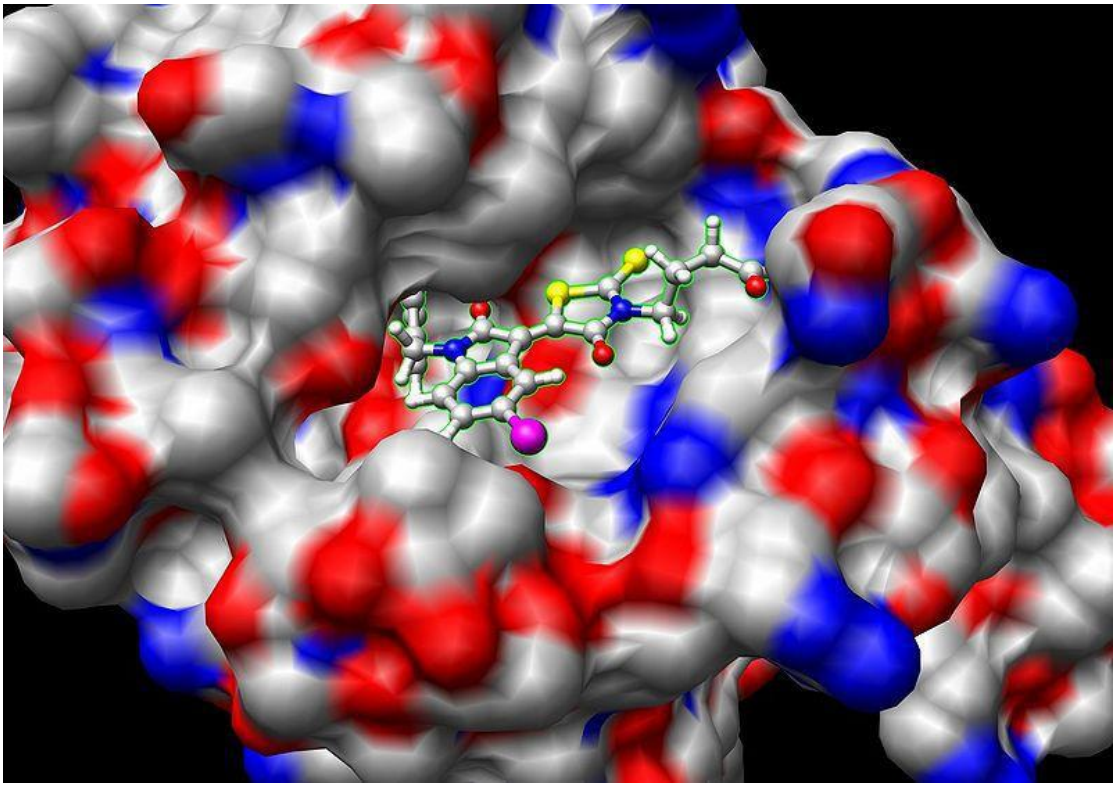


Figure (1.18) : Molecular docking of ligand to a protein receptor to produce a complex.

1.2.14.2 Types of Docking:

a) Rigid body docking, where both the receptor and small molecule are treated as rigid

b) Flexible ligand docking , where the receptor is held rigid, but the ligand is treated as flexible.

c) Flexible docking, where both receptor and ligand flexibility is considered

The most commonly docking algorithms use the rigid receptor/flexible ligand model.

The principle docking methods that are used extensively employ search algorithms based on Monte Carlo, genetic algorithm, fragment-based and molecular dynamics.

Some programs that are well-suited for high throughput docking of a large database of molecules include: DOCK , FlexX , GOLD and ICM (Abagyan ,*et al*.,1994).

1.2.14.3 Pre-Docking Compound Filtering:

Prior to carrying out docking calculations, it is beneficial to pre-select the database of compounds to be docked by applying hierarchical filters to

produce 'focused'database.

Such filters often drastically reduce the number of compounds that need to be evaluated in the more demanding docking calculation.

Starting from a set of 90, 000 compounds, the successive application of 2-D substructure queries to identify known metal-binding groups, 3-D pharmacophore based queries and flexible superposition reduced the database to 100 compounds.

Docking calculations of the selected 100 compounds with FlexX identified four potent inhibitors with activities in the nanomolar range. Subsequent crystallographic studies confirmed the predicted docking poses of two of these hits (Gruneberg,*et al*.,2002).

In general case, a suitable set of pharmacophores can be derived by performing a binding site analysis to identify regions of favorable

protein-ligand interactions. An excellent review has been published describing the application of pharmacophore based modeling methods in

discovering new leads in the absence of structural data.

A hybrid approach in which initial pharmacophore-based filtering was followed by subsequent docking of a small subset of compounds yielded novel inhibitors of alanine racemaseand dihdyrofolatereductase (Rastelli,*et al*.,2003) .

1.2.14.4 Mechanics of Docking:

To perform a docking screen, the first requirement is a structure of the protein of interest. Usually the structure has been determined using a biophysical technique such as x-ray crystallography, or less often,

NMRspectroscopy. This protein structure and a database of ligands serve as inputs to a docking program.

The success of a docking program depends on two components such as search algorithm and scoring function (Davis,*et al*.,2003).

1.2.14.4.1 Searching Conformational Space :

The search space consists of all possible orientations and conformations of the protein paired with ligand.

1.2.14.4.2 Scoring Functions :

The scoring function takes a pose as input and returns a number indicating the likelihood that the pose represents a favorable binding interaction, most scoring functions are physics based molecular mechanics force fields that estimate the energy of the pose; a low (negative) energy indicates stable system and thus a likely binding interaction(Rastelli,*et al*.,2003).

An alternative approach is to derive a statistical potential for interactions from a large database of protein-ligand complexes, such as the Protein Data Bank, and evaluate the fit of the pose according to this inferred potential.

There are a large number of structures from X-ray crystallography for

complexes between proteins and high affinity ligands, but comparatively fewer for low affinity ligands as the later complexes tend to be less stable and therefore more difficult to crystallize.

Scoring functions trained with this data can dock high affinity ligands correctly, but they will also give plausible docked conformations for ligands that do not bind. This gives a large number of false positive

hits, i.e. ligands predicted to bind to the proteins that actually do not when placed together in a test tube(Fernandez-Recio ,et al.,2003).

1.2.14.5 Applications Of MolecularDocking :

A binding interaction between a small molecule ligand and an enzyme protein may result in activation or inhibition of the enzyme.

If the protein is a receptor, ligand binding may result in agonism or antagonism.

Docking is most commonly used in the field of drug design. Most drugs are organic molecules, and docking may be applied for:

Hit identification – docking combined with a scoring function can be used to quickly screen large databases of potential drugs in silico to identify molecules that are likely to bind to protein target of interest.

Lead optimization – docking can be used to predict in where and in which relative orientation a ligand binds to a protein (i.e. binding mode or pose). This information may in turn be used to design more potent and selective analogs .

Bioremediation – Protein ligand docking can also be used to predict pollutants that can be degraded by enzymes (Suresh ,*et al*.,2008).

1.3 Objective of the study:

The aim of this study is preparing some cobalamin complexes by using amino acids .

Chapter two

Materials and method

Chapter Two

2. Materials and methods

2.1 Materials:

2.1.1 Methyl cobalamin tablets:500 mcg , IZEK Health care pvt.ltd , Delhi , INDIA .

2.1.2 Alanine: D = 1.421g\cm^3 , 82%

2.1.3 Cysteine: D = 1.23 g\cm^3 , 90%

2.1.4 Chemicals:

Sodium hydroxide,NaOH , 2.1 g/cm^3, 40 g/mole ,97%.

Hydrochloric acid, HCL ,11.8 M,(1.18g /cm^3),36.46 g/mole ,36.5% .

2.1.5 Instruments :

-UV Spectrophotometer , UV -1800 , Shimadzu , Japan .

-Digital polarimeter , SGW $_{ZZ}$-1 , Shimadzu , japan.

-PH Meter , PH sj-4A , Shimadzu , Jaban.

-Water bath , XMTD -702, Shimadzu , Japan.

-Hot plate stirrer , SCOH science , UK .

-Analytical balance .

2.2 Methods of analysis :

2.2.1Preparation of aqua cobalamin :

0.202 g finely ground powder of methyl cobalamin tablets were transferred in to a beaker (100 ml) , 20 ml of deionized water was added , the solution filtered to remove the coated material , the filtrate was transferred to a clean beaker and heated in a water bath at 40 C˚ for 60 min , the pH was measured and recorded.

2.2.2 Preparation of alanine-cobalamin complex :

0.0567 g of alanine weighted and transferred in to a beaker , 20 ml of deionized water was added , the solution checked until complete dissolving , then 10 ml of aquacobalamin was added .

the solution divided in to two beakers :

a) the pH was set at 2 by adding hydrochloric acid (1M) .

b) at pH =12.4 by adding sodium hydroxide (1M) .

the solutions were heated in a water bath at 40 C for 60 min , the colour of solution (a) turned pink **.**

2.2.3 preparation of cysteine -cobalamin complex :

0.177g of cystein weighted and transferred in to a beaker , 20 ml of deionized water was added , the solution checked until complete dissolving , then 10 ml of aquacobalamin was added.

the solution divided in to two beakers

(c) the pH was set at 2 by adding hydrochloric acid (1M).

(d) atpH =12.4 by adding sodium hydroxide (1M).

the solutions were heated in a water bath at 40 C for 60 min , the colour of solution (d) turned weak brown .

2.2.4 UV-vis Analysis :

for all solutions the absorbance was measured in rang of (200-800 nm). by using quartz cell with a path length of 1 cm .

The obtained absorbance was recorded.

2.2.5 Optical rotation measurement :

the values of the optical rotation were measured by using polarimeter

Temp = 29.0 C L = 1.0n =1 wave length = 589.3 nm

Chapter three

Results and discussions

Chapter three

3. Results and Discussion

3.1 preparation step:

Table (3.1) : Set of pH , Temperature and time :

Factors	Alanine-cobalamin complex	Cysteine-cobalamin complex	aquacobalamin
pH	2 , 12.4	2 , 12.4	5.24
Time\ min	60	60	60
Temperature\ C	40	40	40

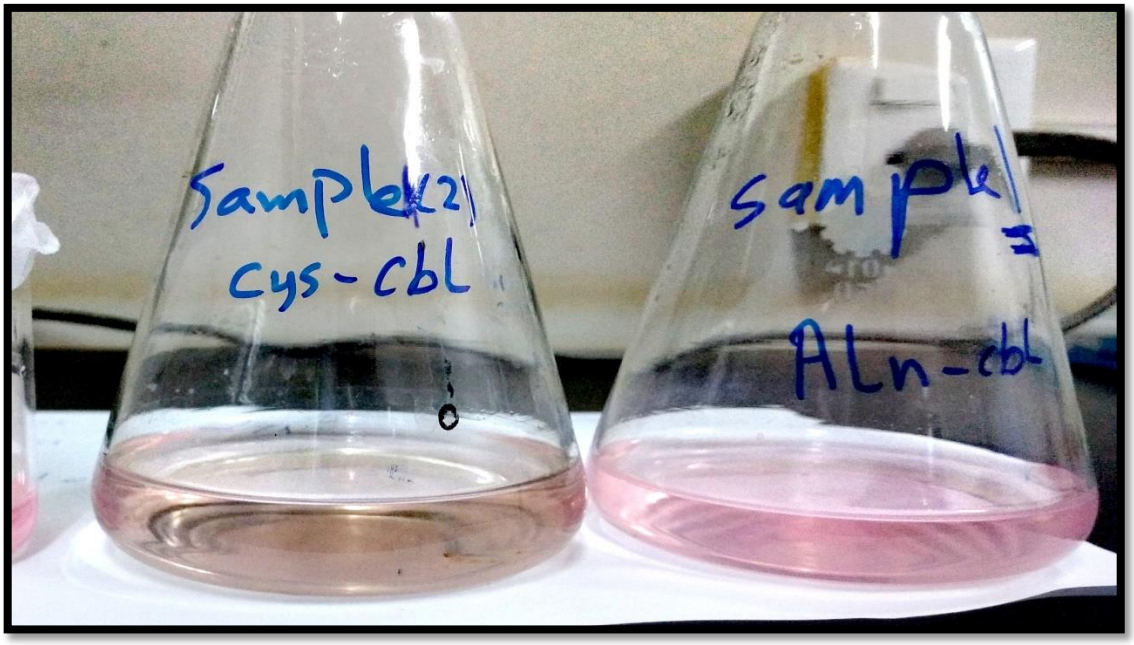


Figure (2.1) : colour of solutions:

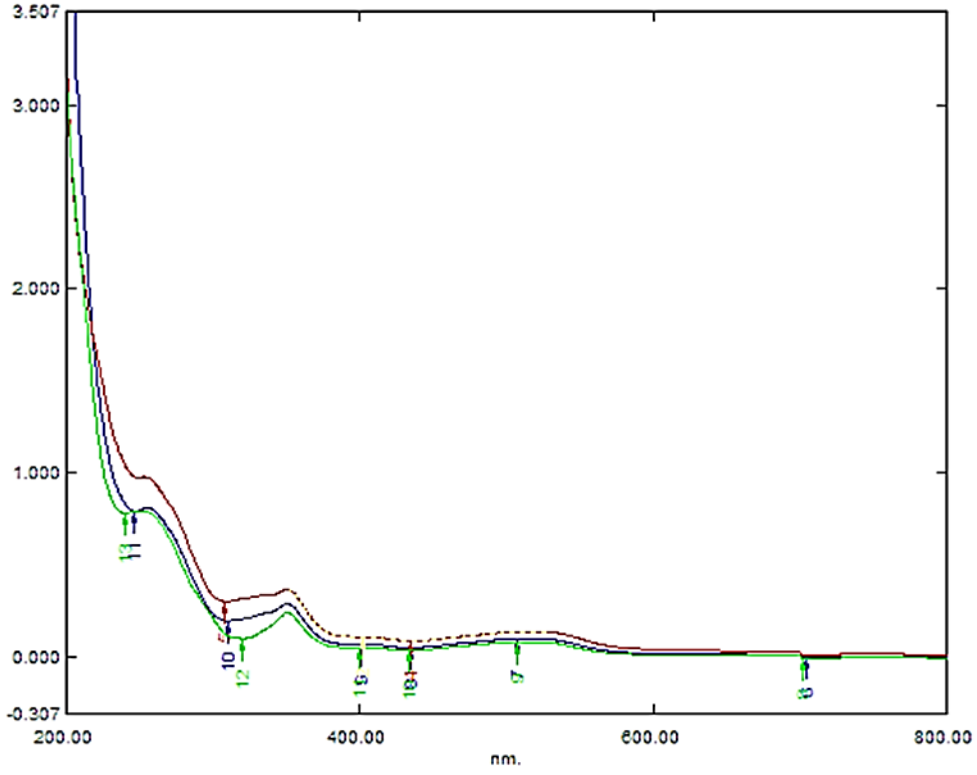
Alanine-cobalamin complex at pH = 2

cysteine-cobalamin complex at pH= 12.2

3.2 UV-Vis analysis :

Table (3. 2) The absorbance of Alanine-cobalamin complex at pH=12.4 , cysteine-cobalamin complex at pH= 2:

NO	Wavelength(nm)	Abs
1	741.00	0.00
2	674.00	0.010
3	523.00	0.080
4	498.50	0.077
5	410.50	0.050
6	350.00	0.243
7	248.50	0.794
8	702.50	- 0.007
9	507.00	0.076
10	433.50	0.038
11	400.00	0.048
12	319.50	0.102



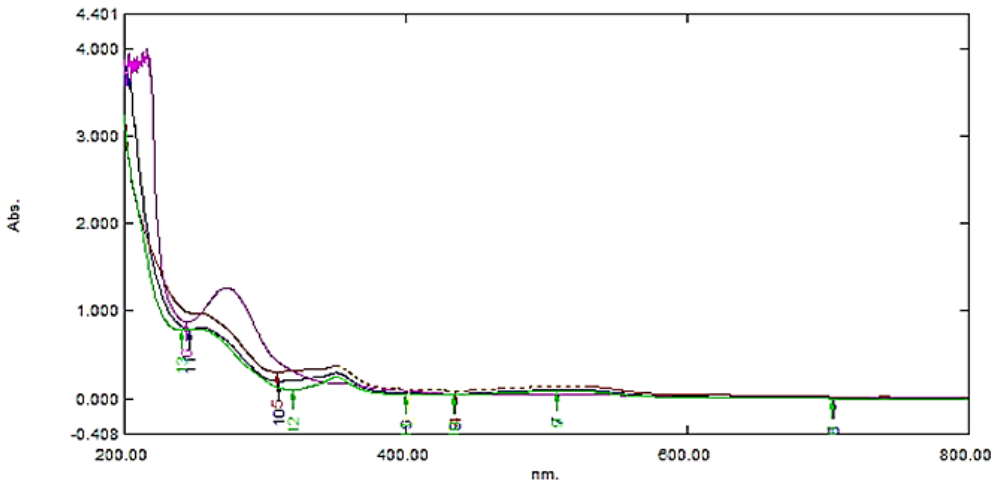
Red=aquacobalamin.

green= cysteine-cobalamin complex at pH= 2 .

blue=alanine-cobalamin complex at pH = 12.4

Table (3.3):The absorbance of Alanine-cobalamin at pH=2 comparing to aquacobalamin :

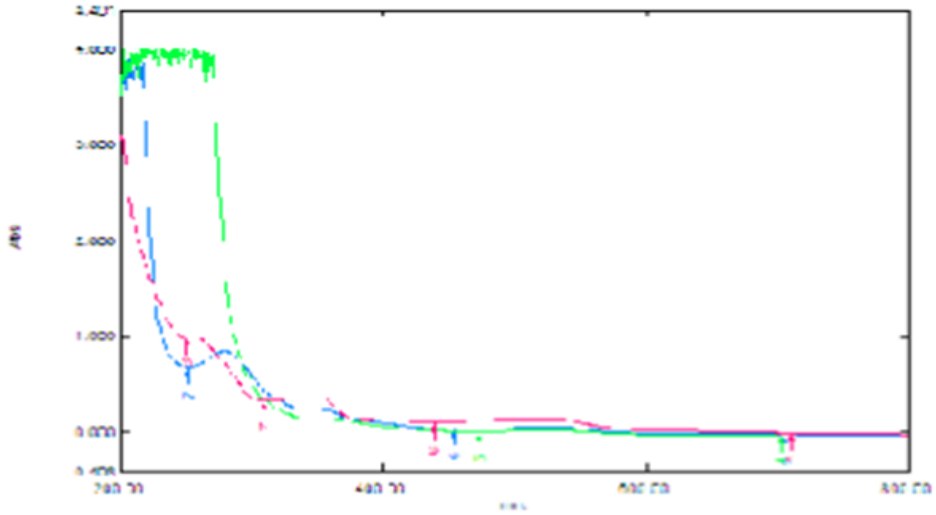
NO	Wavelength (nm)	Abs
1	742.00	0.004
2	699.00	0.012
3	509.00	0.046
4	272.00	1.268
5	702.00	- 0.004
6	244.50	0.874



Violet=alanine-cobalamin complex at pH = 2

Table (3.4): The absorbance of cysteine-cobalamin complex at pH= 12.4comparing to aquacobalamin:

NO	Wavelength(nm)	Abs
1	742.00	-0.002
2	655.00	0.019
3	302.00	4.000
4	703.00	-0.039



Green= cysteine-cobalamin complex at pH= 12.4

Table (3.5) The optical rotation values:

Complex	**Optical rotation(α)**
Aquacobalamin	+ 0.057
Alanine-cobalamin complex at pH= 2	+ 0.031
cysteine-cobalamin complex at pH= 12.4	- 0.100

3.2 Discussion

The complex of alanine-cobalamin was formed at pH= 2 , while in case of using Cysteine the complex formed atpH = 12.4

Heating on a water bath at 40C for 60 minis to increase the rate of reaction .

The release of water from aquacobalamin followed by uptake of the ligands (alanine And cysteine) from solution by the un stable 5-coordinate intermediate.

The results obtained from the UV-vis analysis in the rang 200-800nm showed that difference in spectra between aquacobalamin and alanine-cobalamin complex is little as shown , while it is noticed and clear in cysteine-cobalamin as shown .

Table (3.5) shows that the value of the optical rotation is negative for the cysteine-cobalamin complexat pH=12.4 which equal (– 0.100).

while it is positive for the aquacobalamin and alanine-cobalamin at pH=2 which equal (+ 0.057 and +0.031) respectively .

that means there is a change in the spatial shape of the compound from right to left rotation in case of using cysteine.

Conclusion :

The process of absorbing B12 (cobalamin) involves a formation of a complex between cobalamin and amino acids.

The results from this study on the replacement of the water molecule by amino acids and preparing a cobalamin complexes ledto the following :

the complex between cobalamin and alanine formed in acid medium while it formed in alkaline medium between cobalamin and cysteine .

Alanine-cobalamin complex absorbed at 250 nm and gave an alpha value =+ 0.031,while Cysteine-cobalamine complex absorbed at 300nm and gave an alpha value = - 0.100 .

Recommendation :

- Further Studies could be carried by using molecular docking for cobalamin with amino acids.
- To Study the Quantitative structure activity relationship (QSAR) will be useful.
- Further Studies could be carried by using different types of amino acids with different donor atoms.

References:

Abagyan, R., Totrov, M. and Kuznetsov, D. (1994). ICM—A new method for protein modeling and design: Applications to docking and structure prediction from the distorted native conformation. *Journal of computational chemistry*, **15**(5), 488-506.

Andersen, C. B. F., Madsen, M., Storm, T., Moestrup, S. K. and Andersen, G. R. (2010). Structural basis for receptor recognition of vitamin-B 12–intrinsic factor complexes. Nature, **464**(7287), 445-448.

Banerjee, R. (2006). B12 trafficking in mammals: a case for coenzyme escort service. *ACSchemical biology*, **1**(3), 149-159.

Barker, H. A., Weissbach, H. and Smyth, R. D. (1958). A coenzyme containing pseudovitamin B12. *Proceedings of the National Academy of Sciences of the United States of America*, **44**(11), 1093.

Bleicher, K. H., Böhm, H. J., Müller, K. and Alainine, A. I. (2003). Hit and lead generation: beyond high-throughput screening. *Nature reviews Drug discovery*, **2**(5), 369-378.

Brown, K. L. (2005). Chemistry and enzymology of vitamin B12.*Chemical reviews*, **105**(6), 2075-2150.

Cheng, Z., Li, K., Hammad, L. A., Karty, J. A. and Bauer, C. E. (2014). Vitamin B 12 regulates photosystem gene expression via the CrtJantirepressorAerR in R hodobactercapsulatus. *Molecular microbiology*, **91**(4), 649-664.

Demerre, L. J. and Wilson, C. (1956). Photolysis of vitamin B12. *Journal of the American Pharmaceutical Association* (Scientific ed.), **45**(3), 129-134.

Dereven'kov, I. A., Salnikov, D. S., Silaghi-Dumitrescu, R., Makarov, S. V. and Koifman, O. I. (2016). Redox chemistry of cobalamin and its derivatives. *Coordination Chemistry Reviews*, **309**, 68-83.

Eschenmoser, A. and Wintner, C. E. (1977). Natural Product Synthesis and Vitamin B _12. Science, **196**(4297), 1410-1420.

Fedosov, S. N., Berglund, L., Fedosova, N. U., Nexø, E. and Petersen, T. E. (2002). Comparative analysis of cobalamin binding kinetics and ligand protection for intrinsic factor, transcobalamin, and haptocorrin. *Journal of Biological Chemistry*, **277**(12), 9989-9996.

Feig, M., Onufriev, A., Lee, M. S., Im, W., Case, D. A. and Brooks III, C. L. (2004). Performance comparison of generalized born and Poisson methods in the calculation of electrostatic solvation energies for protein structures. *Journal of computational chemistry*, **25**(2), 265-284.

Fernández-Recio, J., Totrov, M. and Abagyan, R. (2003). ICM-DISCO docking by global energy optimization with fully flexible side-chains. *Proteins: Structure, Function, and Bioinformatics*, **52**(1), 113-117.

Gallo, S., Oberhuber, M., Sigel, R. K. and Kräutler, B. (2008). The corrin moiety of coenzyme B12 is the determinant for switching the btuBriboswitch of E. coli. *ChemBioChem*, **9**(9), 1408-1414.

Gille, D. and Schmid, A. (2015). Vitamin B12 in meat and dairy products. *Nutritionreviews*, **73**(2), 106-115.

Green, R., Allen, L. H., Bjørke-Monsen, A. L., Brito, A., Guéant, J. L., Miller, J. W. and Yajnik, C. (2017). Vitamin B 12 deficiency. *Nature reviews Disease primers*, **3**(1), 1-20.

Gruber, K., Puffer, B. and Kräutler, B. (2011). Vitamin B 12-derivatives—enzyme cofactors and ligands of proteins and nucleic acids. *Chemical Society Reviews*,**40**(8), 4346-4363.

Grüneberg, S., Stubbs, M. T. and Klebe, G. (2002). Successful virtual screening for novel inhibitors of human carbonic anhydrase: strategy and experimental confirmation. *Journal of medicinal chemistry*, **45**(17), 3588-3602.

Halperin, I., Ma, B., Wolfson, H. and Nussinov, R. (2002). Principles of docking: An overview of search algorithms and a guide to scoring functions. *Proteins: Structure, Function, and Bioinformatics*,**47**(4), 409-443.

Hannibal, L., Smith, C. A., Smith, J. A., Axhemi, A., Miller, A., Wang, S. and Jacobsen, D. W. (2009). High resolution crystal structure of the

methylcobalamin analogues ethylcobalamin and butylcobalamin by X-ray synchrotron diffraction. *Inorganic chemistry*, **48**(14), 6615-6622.

Hung, Y., Martin, L. Y., Jackels, S. C., Tait, A. M. and Busch, D. H. (1977). Ring size effects among metal complexes with macrocyclic ligands: synthesis, stereochemistry, spectrochemistry, and electrochemistry of cobalt (III) complexes with unsubstituted, saturated tetraazamacrocycles. *Journal of the American Chemical Society*, **99**(12), 4029-4039.

Koutmos, M., Pejchal, R., Bomer, T. M., Matthews, R. G., Smith, J. L. and Ludwig, M. L. (2008). Metal active site elasticity linked to activation of homocysteine in methionine synthases. *Proceedings of the National Academy of Sciences*, **105**(9), 3286-3291.

Kräutler, B. (2005). Vitamin B12: chemistry and biochemistry. *Biochemical Society Transactions*, **33**(4), 806-810.

Kräutler, B. (2019). Biological organometallic chemistry of vitamin B12-derivatives. In *Advances in Bioorganometallic Chemistry* (399-430). Elsevier.

Kuta, J., Wuerges, J., Randaccio, L. and Kozlowski, P. M. (2009). Axial bonding in alkylcobalamins: DFT analysis of the inverse versus normal trans influence. *The Journal of Physical Chemistry* A,**113**(43), 11604-11612.

Larsson, K. M., Logan, D. T. and Nordlund, P. (2010). ACS Chem. Biol, **5**, 933-942.

Leal, N. A., Park, S. D., Kima, P. E. and Bobik, T. A. (2003). Identification of the human and bovine ATP: Cob (I) alaminadenosyltransferasec DNAs based on complementation of a bacterial mutant. *Journal of Biological Chemistry*, **278**(11), 9227-9234.

Mathews, F. S., Gordon, M. M., Chen, Z., Rajashankar, K. R., Ealick, S. E., Alpers, D. H and Sukumar, N. (2007). Crystal structure of human intrinsic factor: cobalamin complex at 2.6-Å resolution. *Proceedings of the National Academy of Sciences*, **104**(44), 17311-17316.

Murcko, M. A. (1995). Computational methods to predict binding free energy in ligand-receptor complexes. *Journal of medicinal chemistry*, **38**(26), 4953-4967.

Paternain, J. L., Domingo, J. L. and Corbella, J. (1988). Developmental toxicity of cobalt in the rat. *Journal of Toxicology and Environmental Health, Part A Current Issues*, **24**(2), 193-200.

Pino, P., Piacenti, F. and Bianchi, M. (1977). Reactions of carbon monoxide and hydrogen with olefinic substrates: the hydroformylation (OXO) reaction. *Organic Syntheses via Metal Carbonyls*, 2, 180.

Proinsias, K., Giedyk, M. and Gryko, D. (2013). Vitamin B 12: chemical modifications. *Chemical Society Reviews*, **42**(16), 6605-6619.

Randaccio, L., Geremia, S., Demitri, N. and alpers, J. (2010). Vitamin B12: unique metalorganic compounds and the most complex vitamins. *Molecules*, **15**(5), 3228-3259.

Randaccio, L., Geremia, S., Demitri, N. and Wuerges, J. (2009). Structural aspects of B12 chemistry and biochemistry: From simple models to proteins.

Randaccio, L., Geremia, S., Nardin, G. and Wuerges, J. (2006). X-ray structural chemistry of cobalamins. *Coordination chemistry reviews*, **250**(11-12), 1332-1350.

Rastelli, G., Pacchioni, S., Sirawaraporn, W., Sirawaraporn, R., Parenti, M. D. and Ferrari, A. M. (2003). Docking and Database Screening Reveal New Classes of Plasmodium f alciparumDihydrofolateReductase Inhibitors. *Journal of Medicinal Chemistry*, **46**(14), 2834-2845.

Rosenzweig, A. C. (2002). Metallochaperones: bind and deliver. *Chemistry & biology*, **9**(6), 673-677.

Ruetz, M. M., Gherasim, C., Gruber, K., Fedosov, S., Banerjee, R. and Kräutler, B. (2013). Radical Synthesis Opens Access to Organometallic Aryl-Cobaltcorrins-4-Ethylphenyl-cobalamin, a Potential 'Antivitamin-B12'. *AngewandteChemie (International ed. in English)*, ***52***(9).

Rusu, D., Stanila, A., Marian, I. O., Marian, C. O., Rusu, M. and Lucaciu, R. (2009). Synthesis and characterization of some cobalt (II) complexes with amino acids having biological activities. *Rev. Chim*, **60**(9), 939-943.

Sauer, K. and Thauer, R. K. (1999). The role of corrinoids in methanogenesis. *Chemistry and biochemistry of B12*., 655-679.

Schaffer, L and Verkhivker, G. M. (1998). Predicting structural effects in HIV-1 protease mutant complexes with flexible ligand docking and protein side-chain optimization. *Proteins: Structure, Function, and Bioinformatics*, **33**(2), 295-310.

Schneider, Z. and Stroinski, A. (2011). Comprehensive B12. de Gruyter.

Seiler, H. G., Sigel, H. and Sigel, A. (1988). Handbook on toxicity of inorganic compounds.

Siega, P., Wuerges, J., Arena, F., Gianolio, E., Fedosov, S. N., Dreos, R. and Randaccio, L. (2009). Release of toxic Gd^{3+} ions to tumour cells by vitamin B12 bioconjugates. Chemistry, **15**(32), 7980-7989.

Suarez-Moreira, E., Yun, J., Birch, C. S., Williams, J. H., McCaddon, A. and Brasch, N. E. (2009). Vitamin B12 and redox homeostasis: cob (II) alamin reacts with superoxide at rates approaching superoxide dismutase (SOD). *Journal of the American Chemical Society*, **131**(42), 15078-15079.

Suresh, P. S., Kumar, A., Kumar, R. and Singh, V. P. (2008). An Insilco approach to bioremediation: Laccase as a case study. *Journal of Molecular Graphics and Modelling*,**26**(5), 845-849.

Takeuchi, T., Böttcher, A., Quezada, C. M., Meade, T. J. and Gray, H. B. (1999). Inhibition of thermolysin and human α-thrombin by cobalt (III) Schiff base complexes. *Bioorganic & medicinal chemistry*, **7**(5), 815-819.

Tame, J. R. (1999). Scoring functions: a view from the bench. *Journal of Computer-Aided Molecular Design*, **13**(2), 99-108.

Thompson, J. P. and Marrs, T. C. (2012). Hydroxocobalamin in cyanide poisoning. *ClinicalToxicology*, **50**(10), 875-885.

Vallee, W. M. B. L. (1993). [3] Cobalt as probe and label of proteins. *Methods in enzymology*, **226**, 52-71.

Varadinova , T., Shishkov, S., Panteva, M. and Bontchev, P. (1996). Effect of Complexes of Cobalt With Aminoacids on the Replication of Herpes Simplex Virus Type 1 (HSV-1). *Metal-based drugs*, **3**(3), 149-154.

Viola-Villegas, N., Rabideau, A. E., Bartholoma, M., Zubieta, J. and Doyle, R. P. (2009). Targeting the cubilin receptor through the vitamin B12 uptake pathway: cytotoxicity and mechanistic insight through fluorescent Re (I) delivery. *Journal of medicinal chemistry*, **52**(16), 5253-5261.

Watanabe, F. (2007). Vitamin B12 sources and bioavailability. *Experimental biology and medicine*, **232**(10), 1266-1274.

Williams, R. J. P. and Da Silva, J. F. (2005). *The chemistry of evolution: the development of our ecosystem*. Elsevier.

Yamada, R., Shimizu, S. and Fukui, S. (1968). Disproportionation of vitamin B12 under various mild conditions. *Biochemistry*, **7**(5), 1713-1719.

Yamani, L. (2008). *Studies on transcobalamin in cultured fibroblasts from patients with inborn errors of cobalamin metabolism* (Doctoral dissertation, McGill University).

Zagalak, B. and Friedrich, W. (Eds.). (2019). Vitamin B12: Proceedings of the 3rd European Symposium on Vitamin B12 and Intrinsic Factor, University of Zurich, March 5–8, 1979, Zurich, Switzerland. Walter de Gruyter GmbH & Co KG.