Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 1, 2015

Original Article

EFFECT OF ADDITIVES ON INTRANASAL PREPARATION OF CYANOCOBALAMIN

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Received: 08 Oct 2014 Revised and Accepted: 10 Nov 2014

ABSTRACT

The following study involves formulation and evaluation of intranasal preparation of cyanocobalamin. The design of the formula was achieved by preformulation studies of each of solubility, partition, permeation and stability of cyanocobalamin. HPLC (stability indicating assay) wasused only in the stability studies of cyanocobalamin, other parameters were analyzed using a prevalidated spectrophotometric analytical method. In the meantime,such selected adjuncts should have no significant effects on the physical characteristics of the intranasal drops. All components included in the formula were tested regarding their effects on the tested parameter. Also, they were investigated regarding their effect on each parameter tested including the permeation pattern. The latter parameter could be a substitute for in vivo bioavailability, since the data proved that such parameter is highly indicative. Based on the above studies a formula of intranasal drops of cyanocobalamin was designed to contain ingredients suitable for nasal administration. Such formula achieve another important goal which is improving the patient compliance. The formula contains: Cyanocobalamin 0.5%, Sodium metabisulphite 0.5%, β-cyclodextrin 0.5%, Glycerol 10% and Benzalkonium chloride 0.1%. All ingredients were dissolved in a solution prepared at pH value 5.6 using citrate/phosphate buffer.

Keyword: intranasal, cyanocobalamin, partition coefficient, permeation.

INTRODUCTION

Vitamin B12, (Cyanocobalamin), is an essential material for the growth, cell reproduction, hematopoiesis and synthesis of nucleoprotein and myelin. Cells characterized by rapid division (epithelial cells, bone marrow, myeloid cells) appear to have the greatest requirement for cyanocobalamin [1].

Vitamin B_{12} is associated with fat and carbohydrate metabolism as well as protein synthesis [2]. Vitamin B_{12} deficiency results in megaloblastic anemia, gastrointestinal lesions and neurologic damage which begins with an inability to produce myelin and is followed by gradual degeneration of the axon and nerve head [3]. Vitamin B_{12} requires an intrinsic factor-mediated active transport for its absorption [4], therefore, lack of or inhibition of intrinsic factor results in pernicious anemia[5].

Oral absorption of vitamin B_{12} from the gastrointestinal tract (GIT) depends on the presence of adequate intrinsic factor, which is secreted from gastric mucosa. Drugs like the proton pump inhibitors (e. g., omeprazole and lansoprazole) have the potential for interfering with B_{12} absorption, presumably by impairing gastric acid and pepsin secretion, which are thought to be necessary for releasing B_{12} from its protein-binding sites in food [6]. The absorption of vitamin B_{12} is through the formation of vitamin B_{12} -intrinsic factor complex in the stomach following removal of cobalamin from dietary sources. This complex passes to the small intestine where attachment to receptor sites occurs on the ileal mucosa, and vitamin B_{12} is actively transported to portal plasma. Calcium and a pH greater than 6 are required for attachment to the receptor sites [7]. When the receptor sites become saturated, absorption through passive diffusion occurs [8]. Initially, oral doses of B_{12} and intrinsic factor (IF) increase cobalamin levels in patients with pernicious anemia; however, 50% of patients develop intestinal antibodies to IF. Peak plasma levels are attained for oral doses during 8 to 12 hours, in the case of parenteral doses the peak plasma level is attained during 1 hour [9].

Nasal drug delivery has generated widespread interest among the scientific community as an alternative route for the administration of drugs and biomolecules that are susceptible to enzymatic or acidic degradation and first-pass hepatic metabolism [10] which is avoided on using intranasal delivery system.

This can be attributed to the high vascularity and the permeability of the nasal mucosa, which promotes the bypassing processes of the drug and its degradation. The enhanced permeability from nasal mucosa was demonstrated by Hussain et al.[11] when they achieved plasma concentrations of propranolol comparable with those of intravenous concentrations. Cyanocobalamin is passively absorbed though the highly vascular nasal mucosa. Both intranasal forms gel and spray achieve peak serum concentrations within 1-2 hours of administration [12].

The lack of intranasal drops of vitamin B_{12} in the local market is the main driving force to investigate and formulate intranasal drops of cyanocobalamin. Cyanocobalamin permeation from the nasal cavity is reported [13-15] by using gel or spray.

Such type of dosage form is not familiar with most people, although it provides easily administrable drug. But still the nasal drops are the major dosage forms applied to the nose and accepted by patients.

In the literature, several methods of analysis are reported including microbiological assay [16], spectrophotometry [17], chemi luminescence [18], atomic absorption spectrometry [19], capillary electrophoresis [20]and high pressure liquid chromatography (HPLC) [21] have been proposed for the determination of vitamin B12. The latter method is the highest sensitive and reproducible method, and it is considered as the stability indicating assay for vitamin B12 dosage forms.

MATERIALS AND METHODS

Materials

Cyanocobalamin, vitamin B_{12} {obtained from The Chemical Industries Development "CID" company, Egypt}, Sodium metabisulphite, Sodium sulphate, Sodium Ascorbic acid, Dimethyl Formamide, Carboxypropylmethylcellulose, Citric acid monohydrate and Hydrochloric acid {El nasr pharmaceutical chemicals co., Egypt}, β-cyclodextrin{gifted from Medical Union Pharmaceuticals "MUP", Egypt}, Dimethyl sulphoxide,

N-Octanol{Mumbai, India}, Glycerol {pkd. By: El Gomhouria Co., Egypt}, Hydroxypropylmethylcellulose{Gifted from Global Napi Pharmaceuticals "GNP", Egypt}, Benzalkonium chloride{China}, Disodium Hydrogen Phosphate, Sodium hydroxide{Riedel-de Haën, Germany, Sigma-Aldrich Laborchemikalien GmbH}.

Methods

1) Determination of solubility pattern of Cyanocobalamin

An excess of cyanocobalamin powder (exactly weighted 500mg), were placed in screw capped tubes containing 10 ml of purified water wrapped with aluminum foil. The tubes were rotated 50 rotation per minute (rpm) in constant temperature water bath at 30±0.5 ºC. The determination of the amount dissolved was achieved at various time intervals for a period of three hours. At each time interval the whole content of the bottle was filtered through sintered glass filter no. 40. An exactly measured aliquot was drawn from the clear filtrate and suitably diluted with distilled water and subjected to the spectrophotometric determination. Saturated solution of cyanocobalamin (constant readings) was mostly obtained after thirty minutes. This time was specified by comparing the concentration of cyanocobalamin at various time intervals for three hours, and found that after thirty minutes no increase in the concentration of cyanocobalamin could be detected. The effect of pH value of cyanocobalamin solution was studied using the previously described procedure. The pH values tested were 2.2, 3, 4, 5, 6, 7 and 7.8; the buffer used was citrate phosphate buffer (MacIlvaine's buffer)[22]. At each pH value the cyanocobalamin content was determined three times and the average was calculated. The standard deviation and error were calculated for each determination. The effect of polarity on cyanocobalamin solubility was tested. Cyanocobalamin exactly weighted 500mg was added to 10 ml of each mixed solvent shown after:

- Purified water: Ethanol {(80:20), (70:30), (50:50), (40:60) and (20:80)}.(high polarity)
- Purified water: propylene glycol {(80:20), (70:30), (50:50), (40:60) and (20:80)}.(Intermediate polarity)
- Purified water: Glycerol {(80:20), (70:30), (50:50), (40:60) and (20:80)}.(lowest polarity tested)

The previously described methodology was applied.

2) Determination of Partition coefficient of cyanocobalamin (N-Octanol/purified water)[23]**.**

The determination of partition coefficient of cyanocobalamin was determined. Then a certain concentration of cyanocobalamin (12 mg/ml) in MacIlvaine's buffer at different pH values (3, 4, 5, 5.6, 6, 7 and 7.8) was prepared to study the pH effect.

The effect of the formulation components on cyanocobalamin partition coefficient was studied using instead of the aqueous phase a solution of either sodium metabisulphite, glycerol, benzalkonium chloride or β-cyclodextrin. The effect of each of such component was investigated separately at concentrations of 1.0 %, 2.0 % and 5.0% for each component.

3) Determination of Cyanocobalamin Permeation Pattern

The main idea applied is subjecting cyanocobalamin in solution to a certain stress (gravity) to penetrate a semi-permeable membrane in Permeation Cell designed by the authors, lower compartment 63 mm×70 mm×26 mm in contact with the upper compartment by a hole 16 mm×17 mm (c. f. picture of the cell fig. 1) Membrane Disc Mixed Cellulose {DI: 47 mm, pore size: 0.47 µm}.

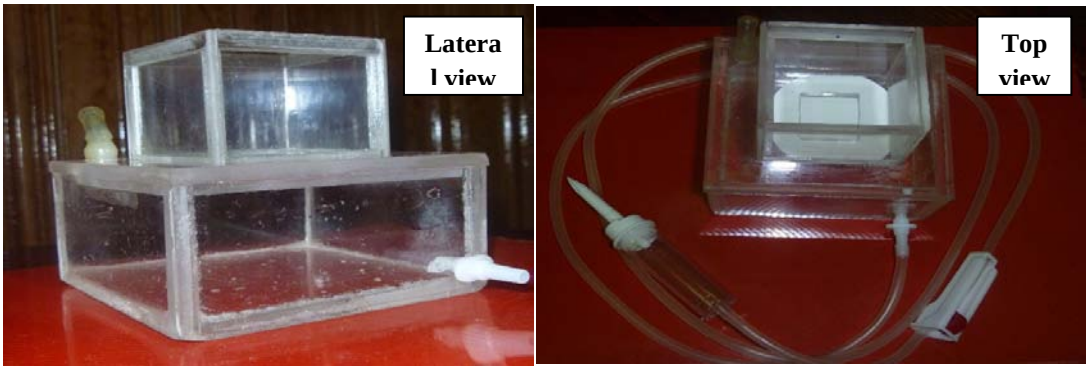

Fig. 1: Permeation Cell; in the left lateral view, while the right is top view

And receiving by flowing aqueous medium (pH 7.0) to imitate the in-vivo condition. A solution of cyanocobalamin (12mg/ml) was prepared in purified water at pH 7.0; One ml from the prepared solution was delivered to the upper surface of the semi-permeable membrane. The volume of the aqueous solution in the lower compartment was 170 ml. Such liquid was moved to the draining container at a rate of 120 drops/min. The liquid drained was collected every 15 sec and determined separately. The time of determinations was adjusted to the following time intervals 15, 30, 45, 60, 90, 120, 150 and 180 seconds. The accumulated data for each time interval were calculated. The fluid temperature was adjusted to 37±0.5C° by immersing the lower compartment of the apparatus in a thermostatically controlled water bath and the samples were subjected to spectrophotometric determination.

The influence of pH of cyanocobalamin solution located at the upper compartment was investigated using the previously described methodology. The pH values tested were 2.2, 3, 4, 5, 6 and 7 using MacIlvaine's buffer[22].

The effect of the presence of polymer with the cyanocobalamin solution was tested using the method applied before. The polymers investigated were propylene glycol, glycerol and polyethylene glycol 400. Each determination was carried out using 20% of the tested solvent and mixed with the aqueous solution in which cyanocobalamin was dissolved (12mg/ml).

The influence of various electrolytes on cyanocobalamin permeation behavior was carried out by applying the same procedures mentioned above. The electrolytes tested were sodium chloride, potassium

chloride, sodium stearate, calcium chloride, sodium sulphate and sodium metabisulphite; In a concentration of 5% w/v.

The determination of the permeation patterns of cyanocobalamin nasal formulation (5mg/ml) was carried out using the previously applied procedure. The tested materials were namely sodium meta-bi-sulfite (0.5%), β-cyclodextrin (0.5%), glycerol (10%), benzalkonium chloride (0.1%), such solution was prepared at pH value 5.6 using citrate/phosphate buffer.

4) Preparation of various Cyanocobalamin Formulations

Cyanocobalamin solutions (5mg/ml) were prepared containing either benzalkonium chloride (1%), sodium sulfite (5%), sodium ascorbate (5%), sodium bi-sulfite (5%), sodium meta-bi-sulfite (5%), dimethylsulphoxide (1%), dimethylformamide (1%), β-cyclodextrin (1%), glycerol (10%), propylene glycol (10%), hydroxypropylmethycellulose (10%) or carboxymethylcellulose (10%). The effect of pH was investigated by preparing cyanocobalamin (5mg/ml) dissolved in MacIlvaine's buffer at different pH values (2.2, 3, 4, 5, 6 and 7).

5) Stability Determination of Cyanocobalamin at various Formulations Components

Each solution previously described was adjusted to contain one component only of the formulation of cyanocobalamin nasal drops. The prepared solutions (each group) were divided into 15 equal portions (5 ml each). Each portion was quantitatively transferred to 10 ml glass brown ampoules, and then the ampoules were sealed. The sealed ampoules were wrapped by aluminum foil to overcome the detrimental effect of the light. This procedure was applied to simulate the shelf life and the influence of the temperature was the only stress factor studied by this procedure. The wrapped sealed ampoules were stored in a thermostatically controlled stability cabinet and subjected to three levels of temperature (30, 40, and 60˚C). The storage time was extended to fourteen days. At each of 1, 2, 3, 7 and 14 days intervals one ampoule from each solution was subjected to analysis applying HPLC technique.

The proposed formula was subjected to the previously mentioned conditions of storage and analyzed using HPLC technique.

RESULTS AND DISCUSSION

Influence of pH values on solubility patterns of cyanocobalamin

The standard deviation is 0.0424 and the variance is 0.0018, results obtained are presented in fig. (2)

From the fig. it is obvious that the amount of cyanocobalamin converted to molecular size are nearly constant during all pH values tested. This indicates that cyanocobalamin cannot be involved in acid/base reaction [24].

A sight into molecular configuration of cyanocobalamin it is noticed that cyanide group and cobalt attached by weak bonds to one of the nitrogen pentane structure. While the peripheries of the molecule are presented by several oxygen and adjacent imino or amino group as well as OH group. The presence of both groups potentiates a condition suitable to form intermolecular hydrogen bonds which has an effect on limiting its solubility, in the meantime, such configuration is highly complexed and the slight availability of free active group at the exposed surface of the molecule of cyanocobalamin which get in part reaction with surrounding anion and cation of water at different pH values. This postulation is nearly acceptable and supported by solubility results obtained.

Influence of polarity of the media on solubility of cyanocobalamin

The solubility of cyanocobalamin was determined at three different polar systems which comprises high polarity such as water (ε = 80). Gradual decrease of polarity is achieved by preparing various ratios of water mixtures with either glycerol (ε = 42.5), propylene glycol (ε = 32.1) or ethanol (ε = 24.3)[25] these were used as solvents for testing cyanocobalamin solubility.

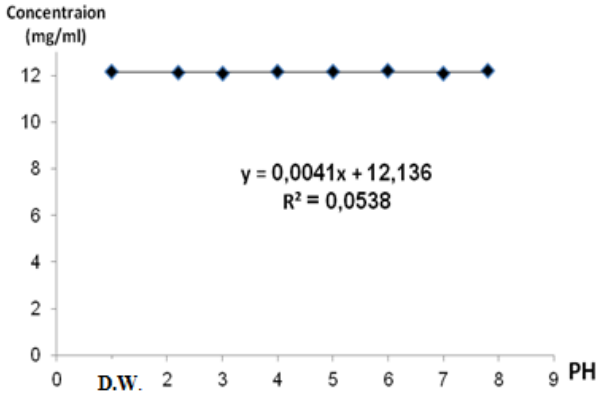

Fig. 2: Solubility of cyanocobalamin at various pH values

The dielectric constant is one of the most important properties of the mixed solvents, which controls and enhances its various applications [1][26]-[28][1]. In pharmaceutical and analytical sciences the dielectric constant of mixed solvents is required to predict the solubility and chemical stability of the drug [1][29]-[33][1]. The results are graphically illustrated in fig. (3):

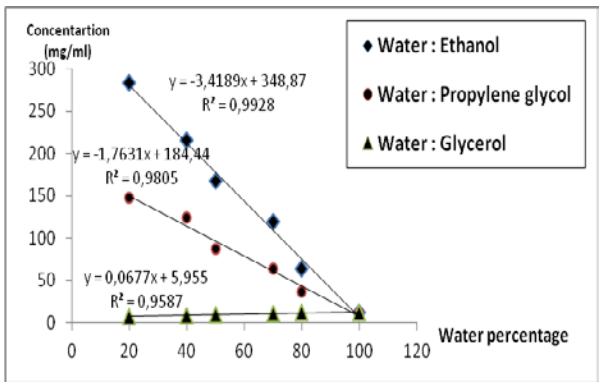

Fig. 3: Solubility of cyanocobalamin at various polar systems

The fig. shows that the least solubility of cyanocobalamin is found in the case of water-glycerol system, while the drug solubility is relatively increased in the case of water-propylene glycol system, and the maximum solubility was observed in the case of water-ethanol system.

The results indicate that the polarity is not the sole factor affecting the solubility of cyanocobalamin; another factor may have a significant effect. This factor is most probably the effect of the components of the system on the solubility away from polarity. This assumption is proved by comparing the solubility of cyanocobalamin in the three systems tested. The influence of such systems on the solubility of cyanocobalamin is mainly dependent on the effect of each of ethanol, propylene glycol and glycerol on the solubility of cyanocobalamin. On comparing the obtained data of each system, a second stress factor other than polarity has a significant effect on the solubility pattern of cyanocobalamin. Such other factor is the solubility influence of ethanol

only or propylene glycol only on the drug molecule. Based on this postulation the solubility of cyanocobalamin is high in presence of high percentage of ethanol. This proved that cyanocobalamin is soluble in ethanol more than propylene glycol. This is easily understandable on considering that ethanol as a molecule has a significant effect on the solubility of cyanocobalamin (B. P. 2007).

In other words, such increase may be attributed to the influence of pure solvents (alcohol, propylene glycol) on the solubility of cyanocobalamin. Based on the above mentioned facts the latter influence of pure solvents on the solubility of cyanocobalamin may be superior in effect regarding the amount of solvent rather than the polarity effect.

In the meantime, glycerol is not reported as a solubilizer for cyanocobalamin (B. P. 2007). Which indicatesthat glycerol decreases the solubility of cyanocobalamin in its water/glycerol mixture; the results is opposite with the above mentioned results in the case of ethanol and propylene glycol. In this case the results obtained coincide with the previously proved indirect relation of polarity and solubility of cyanocobalamin. Accordingly, the polarity is not the only factor affecting solubility of cyanocobalamin.

Partition behavior of cyanocobalamin between N-octanol and purified water

The influence of concentration of cyanocobalamin in aqueous phase on its tendency to migrate towards organic phase was studied. Neglected changes observed on changing the initial concentration of cyanocobalamin in the aqueous phase within the range of 1-12 mg/ml. The mean partition is 0.02556, with standard deviation of 0.000183 and the variance is 0.00 for this ratio.

Based on these observations it was found that the migration attitude of cyanocobalamin molecule to the organic phase is nearly constant. This could provide a clue for nasal absorption [34].

At different pH values

The influence of pH of the aqueous phase on partitioning of cyanocobalamin to non-aqueous phase was investigated. The results obtained are graphically illustrated as histograms in fig. (4)

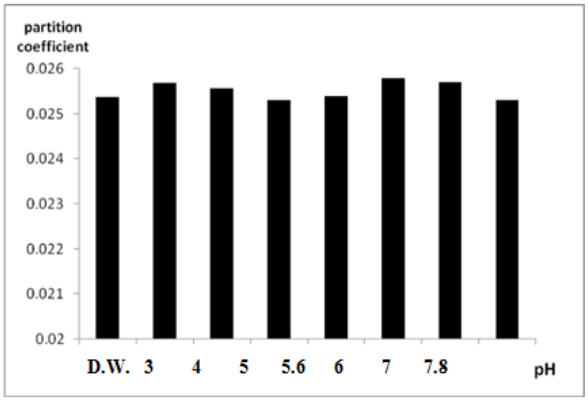

Fig. 4: Partition coefficient of cyanocobalamin at various pH values

From the fig. it is clear that the pH value of the aqueous media has no influence on the partition behavior. The mean equals 0.02552 and its standard deviation is 0.0002. This result indicates that the rule of pH value on the cyanocobalamin in solution is almost zero.

With different components of nasal drop

The components tested include sodium metabisulphite, glycerol, β-cyclodextrin and benzalkonium chloride. The results obtained are graphically illustrated in fig. (5):

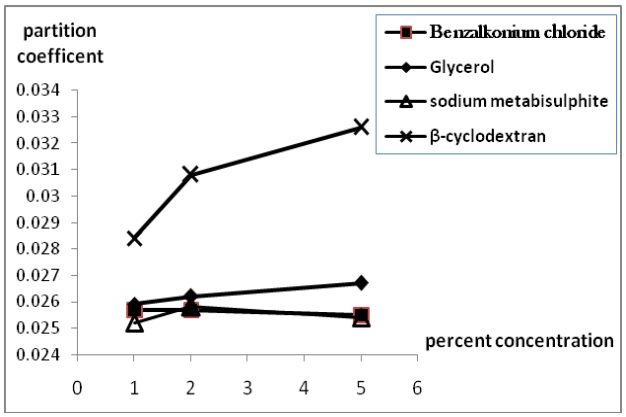

Fig. 5: Effect of added substances on partition coefficient of cyanocobalamin

From the Figure, at zero concentration of each component the partition coefficient of cyanocobalamin alone is mainly (0.0255) {oil/water} such value increases in presence of β-cyclodextrin (1%) and almost slight increase was observed in the case of benzalkonium chloride and glycerol while a slight decrease was observed at lowest concentration of sodium metabisulphite.

According to the previously mentioned observations at lowest concentration (1%) of the components a decreasing order of β-cyclodextrin > glycerol benzalkonium chloride > sodium metabisulphite. This arrangement indicates that the presence of β-cyclodextrin even in a low concentration has a role in increasing the rate of migration of cyanocobalamin molecule to the oily phase.

At the highest concentration of the tested substances the arrangement in the same order. Most probably the increase of the partition coefficient of cyanocobalamin is attributed to complex formation between cyanocobalamin and β-cyclodextrin [35-39]. The presence of glycerol alone proved its slight or minor effect on increasing the migration of cyanocobalamin to the organic phase as revealed by this experiment.

Finally, Benzalkonium chloride and sodium metabisulphite are proved that they have equal effect on partition behavior at the highest concentrations tested. Such result could be explained on the basis that both benzalkonium chloride and sodium metabisulphite are ionic compounds [40]. The presences of such high charged anion or cation are responsible for the formation of a stronger bond with cyanocobalamin than that exists between cyanocobalamin and water molecule.

Permeation behavior of Cyanocobalamin through semipermeable membrane

The permeation characteristics of cyanocobalamin from unsaturated solution (12mg/ml) through membrane matrix (pore size 0.45µm) were studied. Such permeation behavior can be evaluated using the previously described permeation cell [41].

Table 1: Permeation pattern of cyanocobalamin (12.02mg/ml)

Time (Sec.)	Amount permeated (mg)	Percent permeated (%)
15	1.54	12.8
30	2.04	17
45	3.13	26
60	4.05	33.7
90	5.98	49.7
120	7.59	63.2
150	9.18	76.4
180	12	99.82
Slope 0.5164		
Regression coefficient 0.9938		

Results from table (1) indicate that 99.82% of cyanocobalamin initial concentration (12mg/ml) penetrated through the used membrane to the flow solution in the lower compartment and this was achieved within 180 seconds; which encourage the use of cyanocobalamin as nasal drops.

At different pH values

Absorption of compounds that cross biological membranes and mucosal barriers may be affected by the hydrophobic/hydrophilic balance of the compound, and for weak acids or bases, by the pH of the environment. For nasal mucosal membranes, a range of studies evaluating the effect of lipophilicity and pH on nasal absorption has been performed [42,43].

The influence of the pH values of cyanocobalamin solution on the extent of permeation was investigated at 150 sec. this value was chosen on the basis of differences of permeation to be clearly noticed. The results are presented by histograms in fig. (6):

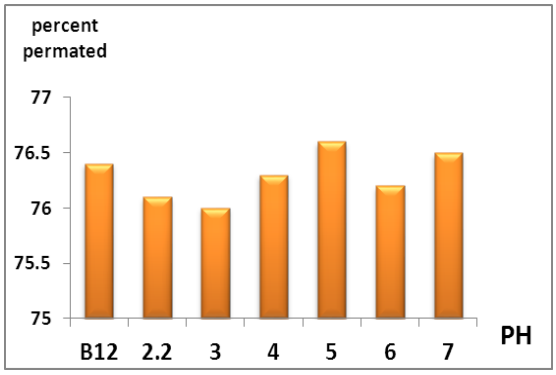

Fig. 6: Effect of different pH values on permeation pattern of cyanocobalamin

In the fig. the permeated quantity of the drug increased with the increasing pH values till pH 5.0. After this a decreasing behavior is noticed till pH 7.0. During this order a certain decrease of the permeated amount was obtained at pH 3, and pH 6.0. At these two pH values the capability of water to hold cyanocobalamin molecules is significantly increased. Such increase in the holding capability exerted between cyanocobalamin and water molecules resulted in decrease of the permeation pattern through the membrane. Collectively the permeation magnitude of cyanocobalamin is maximum at pH 5.

At different polymers

The permeation magnitude of cyanocobalamin was determined in presence of three solvents–water mixture with a ratio of 20-80 respectively. The results obtained are illustrated as histograms in fig. (7). The determination was carried out after 150 seconds from starting the permeation experiment.

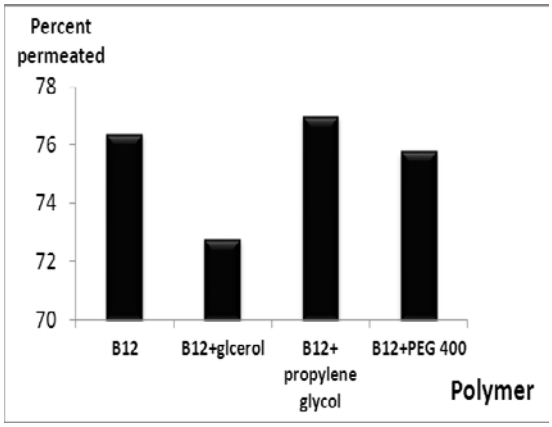

Fig. 7: Effect of polymers on permeation pattern of cyanocobalamin

On discussing the data in the light of strength of holding water molecule to cyanocobalamin, it was found that the results obtained are not coinciding with this assumption. To understand the mechanism of penetration in presence of either glycerol or other two polymers, the concentration of cyanocobalamin in the solution on the upper side of the membrane is the basis of discussion of such unexpected results. Since the permeation of cyanocobalamin follows the rules applied in passive permeation. In this case the concentration gradient is the main force driving the permeation magnitude. In the case of glycerol the solubility of cyanocobalamin is significantly decreased, as previously mentioned, and followed by a decrease of permeation. The reverse is noticed in the case of propylene glycol and polyethylene glycol 400.

At various electrolytes

The influence of various types of electrolytes on the permeated amount of cyanocobalamin was studied. Monovalent electrolytes, divalent electrolytes and surface active agents, all were included in this study. The time of permeation is 150 seconds. The obtained results are illustrated as histograms fig. (8):

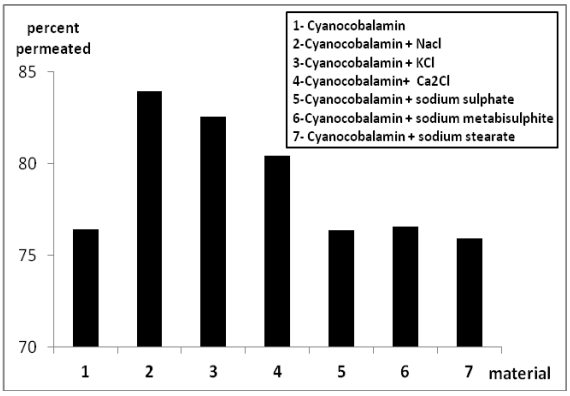

Fig. 8: Effect of various electrolytes on permeation pattern of cyanocobalamin

From the fig. it is clear that all tested electrolytes have an increasing effect towards cyanocobalamin permeation. Sodium chloride has a maximum value regarding the amount of cyanocobalamin permeated.

To explain such results, there are two or more factors driving the permeation magnitude have to be considered. Regarding the valency, such factor is accepted in all cases. On considering the molecular size of the electrolyte tested in combination with valency, it is more sensible to compare all monovalent and all divalent electrolyte tested. Based on the last assumption, the superiority of monovalent over the divalent salts tested is clearly observed. On comparing various monovalent as a function of molecular size, it is found that sodium chloride exhibits lowest molecular weight and highest permeation value. This relation is based on considering the order of permeation magnitude for each of potassium chloride, and sodium stearate. Since this relation is acted on the divalent electrolyte tested, the superiority of calcium chloride compared to the relative lower values in the case of sodium metabisulphite and sodium sulphate is clearly proved.

Permeation patterns of cyanocobalamin formulated as nasal drops

This study was focused on investigating the behavior of cyanocobalamin permeation from the tested formulation. It is shown that the permeation behavior of cyanocobalamin permeated from nasal formulation is more or less similar in the pattern and the magnitude to that from aqueous solution mentioned above, this is proved by comparing the slope and the regression coefficient (R^2) on fig. (9)

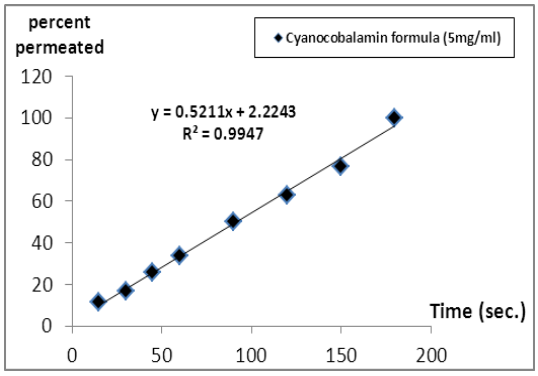

Fig. 9: permeation pattern of cyanocobalamin nasal formulation

With different adjuncts

On trying to relate various materials included in the proposed formula and/or mostly used to formulate the nasal drops. This study was achieved by comparing the obtained slopes in presence of each component with cyanocobalamin at 60℃; such data are graphically illustrated as histograms. Fig. (11) shows that dimethylformamide is characterized by the highest detrimental effect on cyanocobalamin at 60˚C; other components had more or less equal effects on the stability pattern of cyanocobalamin, this effect is found to be characterized by lower slopes compared to the detrimental effect of dimethylformamide (highest slope).

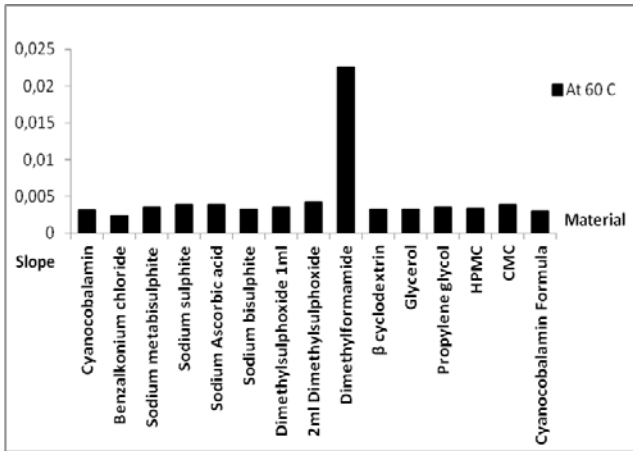

Fig. 11: Slopes of stability pattern of cyanocobalamin with different components

At different pH values

To compare the stability of cyanocobalamin as a function of pH values, the slopes of each pH are graphically illustrated as histogram in fig. (10)

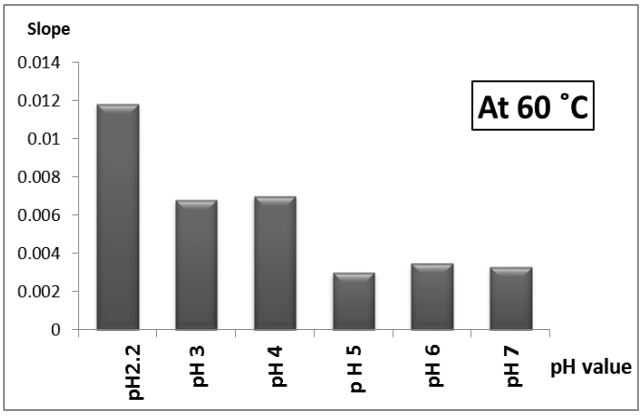

Fig. 10: Relationship between pH values and slope of stability patterns of cyanocobalamin at 60 ºC

From the fig. it is obvious that at pH 2.2 cyanocobalamin exhibit highest slope which indicates highest decomposition rate, while at pH 5 the lowest slope with the lowest decomposition is observed. Based on these results the pH value of the nasal drops formulated should be adjusted to pH range 5:6, in order to provide highest stability value.

Stability pattern of cyanocobalamin at 30, 40 and 60 ˚C[44]

On drawing the percent concentration against time at 30 and 60˚C both have relatively high regression coefficient (R^2), while at 40˚C the straight line is not fitted with the points applied (low regression coefficient). The application of equation related slope and other parameters used to calculate the T_{50} can be applied, the T_{50} at 30˚C is found to be 62500 hrs. (2604 days), and at 60 is equal to 14084.5 hrs. (586.85 days).

According to such equation cyanocobalamin in distilled water proved to have very high stability even at 60˚C, the concentration at 336 hours is equal to 98.8% which indicates long T_{90}.

CONCLUSION

Since the stability of cyanocobalamin in nasal drops in the condition of application was investigated and found to be characterized by higher stability.

The proposed formula is found to fulfill most requirements needed to provide a highly stable cyanocobalamin formulation, and accordingly, proved its suitability as a nasal drop. Using nasal drops provide patient compliance which was one of our targets from this study. Also, direct entry to blood circulation and avoidance of GIT effect, achieved the aim of the study.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGMENT

The authors are grateful for Analytical Chemistry, Tanta University and Suez Canal University for funding the research. Also, CID, GNP and MUP Pharmaceutical Companies for providing the active ingredient.

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