Asian Journal of Chemistry Vol. 18, No. 4 (2006), 2732–2736

Synthesis and Stability of Cyanocobalamine Resinate

S. PALANI*, NISHA MARY JOSEPH, P. K. SHARMA and M.K. GUPTA
Institute of Pharmacy, Bundelkhand University, Jhansi, India
Ph: 09838530648 E-mail: mkgupta@yahoo.com

Cyanocobalamine resinate was prepared by using methacrylate polymers Tulsion-335 and Tulsion-336. The release of the drug from phosphate buffer (pH 6.8) is quite convincing and stable at higher temperature.

Key Words: Cyanocobalamine, Tulsion 335, Tulsion 336.

INTRODUCTION

To reduce the toxicity and to get a better release profile of novel dosage form, alteration in the physicochemical and biological characteristics is needed and this can be achieved by a combination of drugs with appropriate ionic polymer resins to form resinate[1–4]. Cyanocobalamine is used in the treatment of vitamin-B12 deficiency[5]. Cyanocobalamine is photosensitive and in the presence of light the organo-metallic bond is cleaved[6]. So cyanocobalamine in solution can be stabilized by using antioxidants, chelating agents as well as by the resinate formation. The aim of the present study was to load cyanocobalamine on a cation exchange resin and to study the stability and release from its resinate.

EXPERIMENTAL

Cyanocobalamine, tulsion-335 and tulsion-336 were obtained from Supreme Pharmaceuticals and Thermax Ltd., respectively. Potassium dihydrogen ortho-phosphate, disodium hydrogen orthophosphate, citric acid, hydrochloric acid, potassium bromide, sodium chloride and B12 assay medium were supplied by Ranbaxy and Hi Media laboratories and were of analytical/ HPLC grade.

Colorimetric assay of cyanocobalamine

Content of cyanocobalamine was estimated by colorimetric method[7,8]. Pure cyanocobalamine was taken and the solutions were prepared in the concentrations of 5, 10, 15, 20, 25 and 30 mcg/mL by using phosphate buffer (pH 6.8). The absorbance was measured at 361 nm using water as blank (Fig. 1).

Preparation of cyanocobalamine resinate with methacrylic acid polymer

The resins (Tulsion-335 and Tulsion-336) were added to purified water taken in a beaker and mixed using a stirrer for 10 min. The initial pH of the slurry was measured (Table-1).

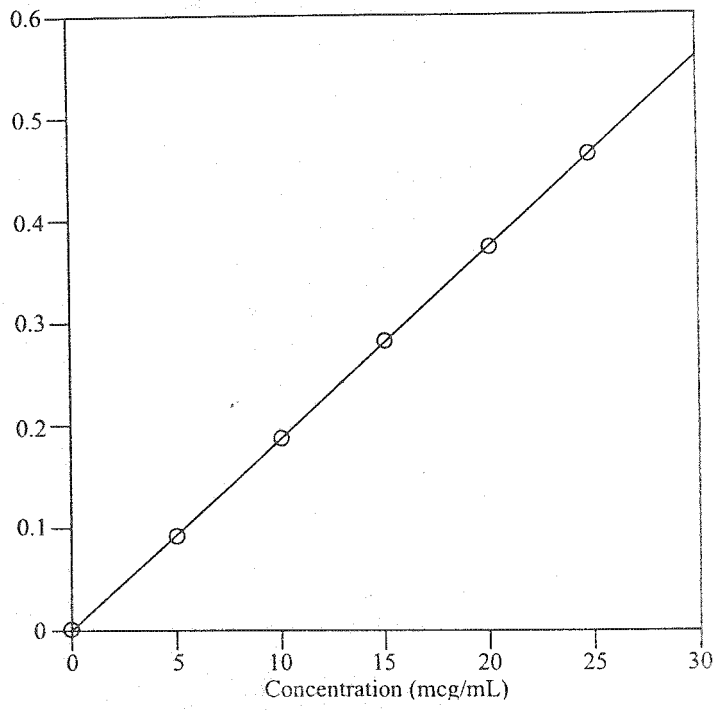

Fig. 1. Calibration graph (cyanocobalamine obeys Beer's law in the range of 5–30 mcg/mL)

TABLE-1
PRELIMINARY STUDIES OF CYANOCOBALAMINE LOADING WITH TULSION 335 AND TULSION 336

Trials	Tulsion-335 (g)	Tulsion-336 (g)	Cyano-cobalamine (mg)	Water (mL)	Initial pH	Final pH	Time (h)	Initial pH adjusted with 20% citric acid	Cyano-cobala mine loaded (%)
1	5	—	50	100	9.44	9.48	2	No	40
2	5	—	50	50	6.00	6.00	2	Yes	46
3	5	—	50	100	4.10	4.08	3	Yes	70
4	—	5	50	100	3.86	3.90	3	No	86
5	—	7.5	50	250	3.80	3.89	3	No	71
6	—	5	50	75	3.88	3.80	3	No	86
7	—	5	50	60	3.70	3.66	3	No	86
8	—	7	50	60	3.69	3.58	4	No	71
9	—	10	100	60	3.78	3.72	5	No	91
10	15	—	150	90	4.01	4.11	5	Yes	81

In some trials, pH was adjusted with citric acid. Cyanocobalamine was added to the slurry and mixing was continued for 2 h. The final pH was also measured (Table-1). The suspension was filtered and the colour of the filtrate ranged from red to colourless. The residue was dried at 45°C for 12 h and the materials were screened through 60 sieve. The dried resinate was analyzed for the content of cyanocobalamine by spectrophotometric method (Table-1).

Loading of cyanocobalamine with Tulsion-335

Cyanocobalamine was loaded in Tulsion-335 as per the ninth trial shown in Table-1 and the method was tested for accuracy and precision by repeating 5 times (Table-2).

TABLE-2
LOADING OF CYANOCOBALAMINE WITH TULSION 335

S.No.	Percentage of cyanocobalamine in tulsion - 335 resinate
1	91.20 ± 0.6228
2	90.00 ± 0.6228
3	91. 00 ± 0.6228
4	89.80 ± 0.6228
5	90.80 ± 0.6228

Release studies of drug cyanocobalamine from cyanocobalamine resinate in different media

100 mg of resinate was accurately weighed and transferred into three 100 mL standard flasks separately. Then, three different media, *viz.*, HCl (pH 1.5), phosphate buffer (pH 5.0) and phosphate buffer (pH 6.8) were added to the three separate standard flasks and made up to 100 mL. All the flasks were agitated using a magnetic stirrer for 45 min. The resulting solution was filtered and assay was done by spectrophotometry. The data are given in Table-3.

TABLE-3
RELEASE STUDIES OF CYANOCOBALAMINE TULSION-335 RESINATE AT pH 1.5, 5.0, 6.8

Tulsion-335	Drug release from resinate in 1.5 pH (%)	Drug release from resinate in 5.0 pH (%)	Drug release from resinate in 6.8 pH (%)
1	22	75	91.0
2	30	77	90.9
3	36	74	90.1

Dissolution studies at phosphate buffer (pH 6.8)

Dissolution studies were carried out *in vitro* using dissolution apparatus II (basket) in a phosphate buffer (pH 6.8) for the cyanocobalamine resinate of Tulsion-335 and was compared with that of gelatin triturate of cyanocobalamine (1%). 900 mL of phosphate buffer (pH 6.8) was used. 900 mg of cyanocobalamine resinate of Tulsion-335, 900 mg of gelatin triturate of cyanocobalamine was added individually and the instrument was set to 100 rpm. Sample was withdrawn after 45 min and cyanocobalamine content was analyzed.

Stabilility studies

The cyanocobalamine resinate with Tulsion-335 and gelatin triturate was kept at temperatures 37° and 45°C for about 8 weeks and the content of drug was determined every week by measuring the absorbance at 361 nm using water as blank.

RESULTS AND DISCUSSION

Cyanocobalamine was stirred with methacrylic acid polymer Tulsion-335 and the loading capacity, the release studies and the stability studies were studied. Dissolution studies were performed for resinate at pH 6.8 since the absorption of cyanocobalamine is maximum at pH 6.8 and it was compared with that of gelatin triturate. Cyanocobalamine resinate-335 showed 91% whereas gelatin triturate showed 31% (Table-4).

TABLE-4
DISSOLUTION STUDIES OF
CYNOCOBALAMINE RESINATE-335 AND GELATIN
TRITURATE FROM PHOSPHATE BUFFER (pH 6.8)

Formulation	Percentage release
Tulsion 335 resinate	91%
Gelatin triturate	31%

The results of stability studies at higher temperatures 37° and 45°C showed that cyanocobalamine resinate showed 4.6% drug loss at 37°C whereas gelatin triturate showed 7% drug loss at 37°C and it was seen that cyanocobalamine resinate showed 3.6% drug loss at 45°C whereas gelatin triturate showed 3% drug loss. This showed that cyanocobalamine resinate is quite stable at higher temperature (Table-5).

TABLE-5
STABILILITY STUDIES OF CYANOCOBALAMINE
RESINATE-335 AND GELATIN TRITURATE

	Cyanoocobalamine resinate-335 (%)		Gelatin triturate (%)	
Initial value	81		114	
Withdrawal week	37°C	45°C	37°C	45°C
1st week	—	—	—	—
2nd week	80	81	110	113
3rd week	80	80.7	110	113
4th week	80	80	110	112
5th week	80	80	110	112
6th week	78	80	110	112
7th week	78	79	110	112
8th week	78	79	107	111

REFERENCES

1. H. Ringsdorf, *J. Polym.*, **51**, 135 (1975).
2. S. Kim and R.J. Peterson, Polymeric Drug Delivery Systems in Drug Design, Vol. X, Ch. 5 (1980).
3. A. Kyclonieus, Controlled Release Technologies: Methods, Theory and Application, CRC Press, Boca Raton (1980).
4. F. Helfferich, Ion Exchange, McGraw-Hill, NewYork, p. 87 (1962).
5. R. Swain, *J. Fem. Pract.*, **41**, 595 (1995).
6. D. Dolphin, Vitamin, Wiley (1982).
7. A.I. Vogel's Text Book of Quantitative Chemical Analysis, 5th Edn. (1982)
8. The Merck Index., Merck & Company, 4th Edn., New Jersey (1976).

(*Received*: 30 July 2005; *Accepted*: 25 April 2006) AJC-4790