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MICROENCAPSULATION OF VITAMIN B-12 BY EMULSION TECHNIQUE

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ABSTRACT

Stability of vitamin B-12 and its uniform mixing in multi-vitamin solid dosage forms was attempted to increase by micro-encapsulating the vitamin by emulsion technique. The prepared products were evaluated for their strength, dissolution rate, and size distribution and compared for the biological stability with various other forms of vitamin B-12. The technique and adopted method were realised to be feasible and therefore, attempts were made for scaling up the procedure to manufacturing scale.

INTRODUCTION

Formulation of solid dosage forms of vitamin B-12 in multi-vitamin products poses two significant problems. Firstly, vit. B-12 stability in these dosage forms has continued to be a major concern. Campbell (1) pointed out from a survey on marketed products that vit. B-12 was below level in all liquid and tablet formulations which was tested by this group. Instability of vit.

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B-12 in presence of other vitamins like ascorbic acid, nicotinamide, thiamine and its interactions with the excipients like talc, sugars, metallic ions etc. in solid dosage forms, has been a proved problem. Stablization of this vitamin with cyanide ions, alpha-hydroxy nitriles (2) and other stablizers has been tried out, but none has gained much success except in some cases where only a limited number of incompatible compatible components were used.

The second problem associated in formulating the solid dosage forms of this vitamin, is content disuniformity due to its minute dose falling in merely micrograms i.e. 5 to 50 micrograms per dose.

These problems are realised to be of much significance and concern on the industrial level which have been observed by these authors in formulating multivitamin tablets and capsules. Microencapsulation of vitamin B-12 on large scale is usually done by spray drying methods, but high initial investment of the equipments and process complexity yield to switch over to a simple and cheaper method.

Method of production of microcapsules has been described. This method was selected after several preliminary experimentations. Microcapsules prepared with varying ratio of shellac and PEG-6000 were selected on the basis of strength and dissolution rate. Selected product was subjected to size distribution and stability studies. Stability of selected product was conducted in comparison with the powedered vitamin, simple trit - urate in mannitol, microcapsules prepared by spray coating and the microencapsulated vitamin B-12 prepared by spray drying. The last product was obtained on request from a manufacturer

and remaining were prepared by the authors.

EXPERIMENTAL

Materials and Reagents. Vitamin B-12 (Roche, Switzerland), PEG - 6000 (Loba-Chem. Indoaustranal Co.), Shellac (B.P.C.), Liquid Paraffin (I.P.), Acetone (Analar), Cylohexane (Sarabhai M. Chemicals), Mannitol (Sarabhai M. Chemicals), Tween 80 (John Baker Inc., U.S.A.) Span 20 (John Baker Inc., U.S.A.).

Preliminary Experimentation. Microcapsules were prepared by the method of Kitajima and Kondo (3) with some modifications. The used concentrations of tween 80 and span 20 as emulsifiers were determined by preliminary experimentation. These concentrations of emulsifiers were found to form a better and more stable acetone/liquid paraffin emulsion.

Selection of coating agents, solvents and washing solvent was made on the basis that the core material, vitamin B-12 and diluent (mannitol) should be insoluble in the solvent (acetone) of coating agents (shellac & PEG-6000), dispersion medium (liquid paraffin) and washing solvent (cyclohexane). Coating materials were selected on the basis of cost, their hardness contribution and dissolution rate of the vitamin from final product.

Mannitol as a diluent was selected since it was found to form more elegant, free flowing, spherical, fine and smooth microcapsules.

Microencapsulation. Microcapsules were prepared by two methods using emulsion technique. The first began with dispersion

of vit. B-12 (one part) in a solution of shellac (58 part) and PEG-6000 (41 part) dissolved in acetone containing tween 80 as an emulsifier. This was emulsified in warm (40^{o}) solution of span 20 (as an complementary emulsifier) in liquid paraffin. The stirring was continued while slowly raising the temperature to $52^{o}$and maintaining it for 30 minutes. Acetone was completely evaporated to precipitate out solid film of shellac and PEG-6000 encapsulating the vitamin to produce fine microcapsules. However, the procedure proved to be lengthy due to evaporation of high amount of acetone required to dissolve large amounts of polymers and therefore the method was superseded by reducing the amount of polymers and hence acetone and using mannitol as a diluent.

1 gm. of vit. B-12 powder passed through 240 mesh and 24 gm. mannitol (100 mesh) were dispersed in the solution of shellac and PEG-6000 (total 75 gms) in acetone (700 ml.) containing 0.5% v/v tween 80. This vitamin dispersion was emulsified by stirring in liquid paraffin (3.0 litre) containing 0.38% v/v span 20. Acetone was evaporated during the process. Subsequently, system was cooled down to ambient temperature under stirring. Microcapsules settled down to bottom when allowed to stand for 30 minutes. Liquid paraffin was decanted off and microcapsules were washed with cyclohexane to remove liquid paraffin and dried at room temperature. Microcapsules were prepared by using varying ratios of shellac and PEG-6000. Microcapsules prepared by this method were fine, free flowing, spherical, shiny and having smooth surface (microphotograph shown in fig. 1).

Triturate of vitamin B-12 for comparison in stability studies was prepared by dissolving the vitamin in alcohol and mixing it

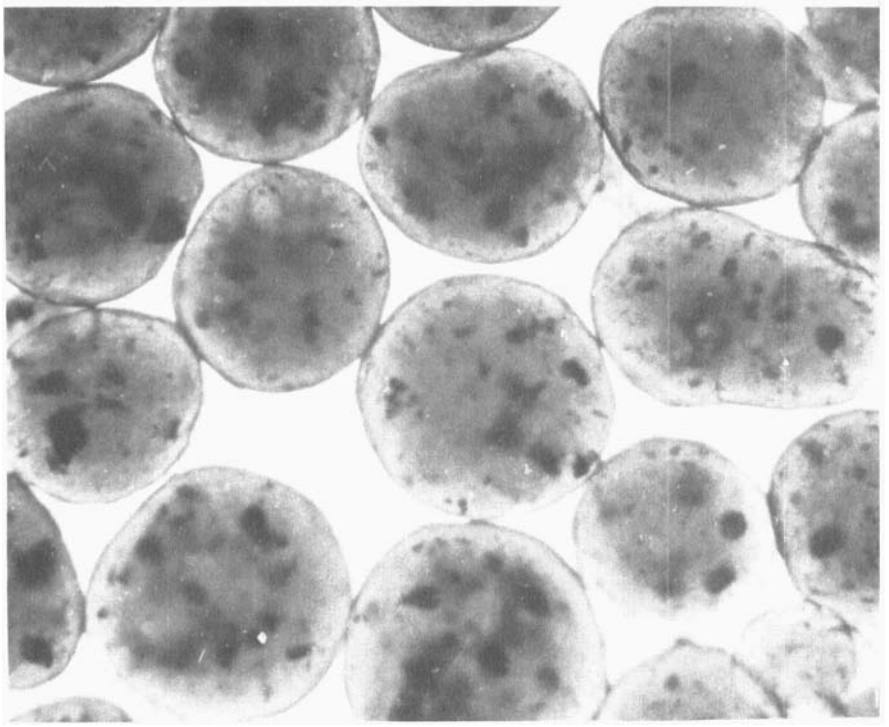

FIGURE-1 Microphoto graphs of vitamin B-12 microcapsules prepared with equal ratio of shellac and PEG-6000 and mannitol. (Magnification X 200)

in 100 mesh mannitol powder. It was then dried at room temperature and passed through 80 mesh seive.

A part of above triturate was coated using ethyl cellulose and PEG-6000 as coating material in Uni-Glatt Machine (Wurster air suspension technique) and stored for the purpose of comparison of stability studies.

RESULTS AND DISCUSSION

Strength of Microcapsules. As the objectives were to use vit. B-12 in multivitamin tablets and capsules, microcapsules should be hard enough to withstand the compaction forces during comp-

TABLE - I

Effect of Compression on Integrity of Microcapsules Prepared with Varying Ratios of Shellac and PEG-6000.

S. No.	Percentage of Shellac:PEG-6000	Condition of Microcapsules after Compression
1.	100 : 0	did not rapture
2.	90 : 10	did not rapture
3.	80 : 20	did not rapture
4.	70 : 30	did not rapture
5.	60 : 40	did not rapture
6.	50 : 50	did not rapture
7.	40 : 60	raptured and deformed.

ression. 5 gms. microcapsules were dispersed in 95 gms., calcium carbonate dummy granules with sufficient lubricants. The compression was carried out in 16 station Cadmack Rotary Machine using four 9 mm. flat bevelled punches at 4 tons/cm^2 pressure. Tablets were disintegrated in water and microcapsules were obtained after straining off the white coloured additives through a fine nylon cloth. Microcapsules were mounted on a slide and observed on 'Vasopan Projection Mi croscope' for the integrity of wall after bearing compression forces. Results are shown in table-I. The products containing higher amounts of shellac were found to possess more strength.

Assay of Vitamin B-12. Vitamin B-12 content in the microcapsules was assayed by USP method (4) at 361 nm using double beam sp-

TABLE - II

Vitamin B-12 Content and Percentage Yield of Various Products.

S. No.	Shellac : PEG-6000	Vitamin B-12 Content(% w/w)	Percentage Practical yield.
1.	100 : 0	0.9874	93.5
2.	90 : 10	1.0806	91.7
3.	80 : 20	0.8934	96.01
4.	70 : 30	0.9344	93.08
5.	60 : 40	0.9987	90.97
6.	50 : 50	1.0030	93.07
7.	40 : 60	1.0506	94.09

ectrophotometer. Results shown in table-II are the average of three estimations.

Percentage Yield. Percentage yield of all products prepared by using different shellac and PEG-6000 ratio was determined by dividing the weight of obtained product by theoretical yield and multiplying the whole by 100. Results are included in table-II. In all the cases the practical yield was found between 90 to 97%.

Dissolution Rate. Steps of absorption of vit. B-12 begins from stomach where 'Intrinsic factor' or transferase enzyme secreted by mucosal cells of stomach binds with it to make it bioavailable (5). Therefore, dissolution studies were carried out to select the product which may release the vitamin with a stipulated time so as to be released in stomach.

D.R. studies were conducted with the USP Apparatus-2 and performed on microcapsules containing 20 mg. of vit. B-12.

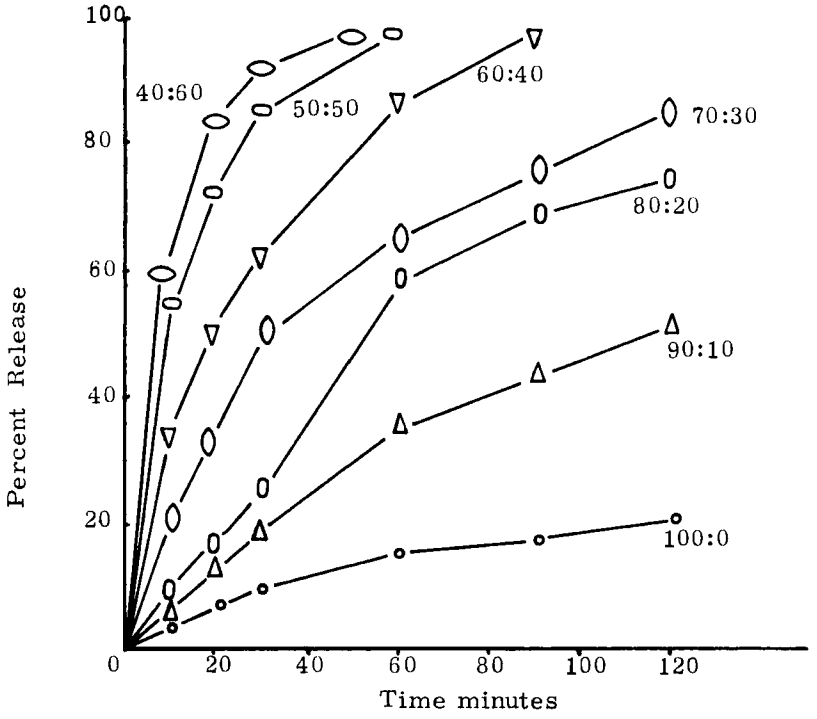

FIGURE-2 D. R. of vit. B-12 from microcapsules prepared with varying ratio of Shellac and PEG-6000.

900 ml. simulated gastric fluid USP was maintained at 37 $\pm$ 1°C and stirred at 50 rpm. 10 ml. of samples were withdrawn at various time intervals and assayed for drug content using double beam spectrophotometer at 361 nm. To maintain a constant volume of dissolution medium, 10 ml. volumes of fresh medium were replaced after each withdrawl. The data shown in fig. 2 are average of triplicate dissolution runs.

PEG-6000 being a water soluble substance forms channels on dissolution and allows the release of drug from microcapsules. Therefore, higher percentage of PEG-6000 showed faster D. R.

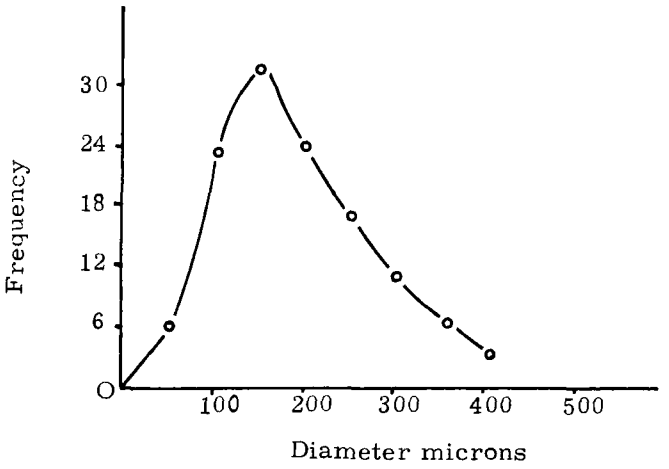

FIGURE-3 Size distribution of microcapsules prepared with equal amounts of shellac and PEG-6000.

On the basis of strength of microcapsules to withstand compression forces and D.R. of vit. B-12, the product prepared with shellac: PEG-6000 (50:50) was selected for further studies.

Microcapsule Size Distributions. The product prepared with Shellac and PEG-6000 (50:50) was sized for microcapsule size distribution using 'Vasopan Projection Microscope'. The microcapsules we prepared followed log normal distribution. It is revealed from the fig. 3 which is a plot between frequency and microcapsule size. The law has been reported to be obeyed by many particulate system including microcapsules (6).

Stability Studies. Stability studies of vitamin B-12 in microcapsules were performed on multivitamin capsules. Each capsule contained : ferrous fumerate 300 mg., thiamine mononitrate 5 mg., riboflavine 5 mg., nicotinamide 50 mg., folic acid 2 mg., vit. B-12-equivalent to 20 mcg, and ascorbic acid 75 mg. For comparative purpose five multivitamin capsule

formulations were prepared containing various forms of vitamin B-12 as following :-

1. Microencapsulated vit. B-12 prepared by emulsification method with Shellac: PEG-6000 (50:50).
2. Pure vit. B-12 240 mesh powder.
3. Spray coated vit. B-12
4. Simple triturate
5. Spray dried vit. B-12 microcapsules

These five preparations were filled in zero size gelatine capsules. Accelarated stability tests showed unpredictable and irregular data therefore stability studies were conducted at room temperature (25 ± 1^{O}) storing the samples in an incubator.

The microbiological assay method (7) was used as stability indicating assay method. Losses of 10% or less were considered insignificant since the microbiological procedure is accurate to $\pm$ 10%.

The stability data plotted in fig. 4 showed first order degradation. The stability of vit. B-12 in five preparations were found in decreasing order as 1, 5, 2, 3 and 4 with half lives of 715, 455, 320, 258 and 136 days respectively. The pure vitamin powder showed much more stability than the triturate and spray coated vit. B-12 triturate but was found less stable than spray dried and microcapsules prepared by emulsification. The probable reason may be explained on the basis of degree of contact of vit. B-12 molecules with the incompatible ingredients. The triturate

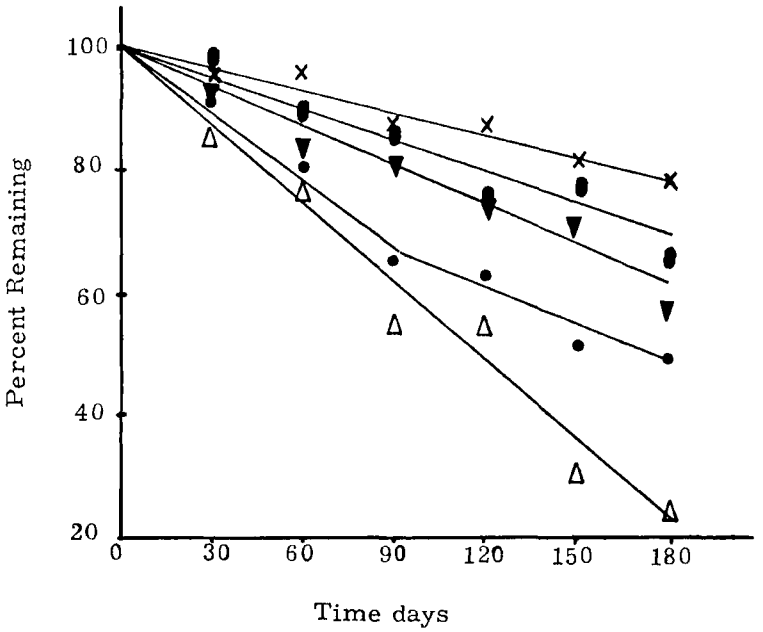

FIGURE-4 Zero order stability plot of various formulations of vitamin B-12. (✖) microcapsules prepared with equal amounts of Shellac and PEG-6000. (●) spray dried. (▼) 240 mesh powder (•) spray coated and (Δ) simple triturate.

showed the poorest stability because the vitamin was spread unprotected on the surface of mannitol, increasing its surface area by several folds. The spray dried product and microcapsules prepared by emulsion technique showed better stability because these were completely protected from degrading agents. The fine powder of vit. B-12 showed better stability than the spray coated and simple triturate because of its comparatively much less surface area and hence much less exposure with incompatible ingredients. The product prepared by Uni-Glatt Machine showed increased stability after three months. Initial fast degradation might be attributed to ununiform coating and coating cracks which allowed the incompatible materials to destroy vit. B-12. However,

after three months, better stability was shown because no unprotected vit. B-12 was left to react further with incompatible materials.

CONCLUSIONS

This method of microencapsulation was found feasible and therefore attempts were made to scale up it to the manufacturing scale. It was possible to scale up the microencapsulation of vit. B-12 by this method to prepare 1% w/w microcapsules of 50 gms. vit. B-12. However, further scaling up of the process needs further studies which are under progress.

ACKNOWLEDGEMENTS

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