Research Article

Bioencapsulation of Probiotic Bacteria by Direct Compression Method

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ABSTRACT

The objective of the present work is to evaluate potential use of compression coating as an alternative method for encapsulation of probiotic bacteria *Lactobacillus acidophilus* to improve their survival when the cells were exposed to an acidic medium. Sodium alginate a biocompatible, natural polymer with pH sensitive gel forming ability has been used as prime coating material. Probiotic cells containing powders were first compressed in to a pellet, which was then encapsulated with a coating material of a combination of sodium alginate and HPMCAS by further compression. Result indicated significant improvement in survival of encapsulated cells when exposed to acidic media of pH 1.2 and 2. The encapsulated cells showed 10^5- 10^6 fold increment in cell survival when compare with free cells under test. The formation of hydrogel barrier by sodium alginate layer has shown to retard the permeation of acidic fluid in to the cells. This contributed to cell survival. In vitro test deduced that release of encapsulated cells in human digestive tract could occur near beginning of colon.

INTRODUCTION

"Probiotics is a live digestible bacterial food supplement, which beneficially affects the host by improving the intestinal microbial balance or by restoring the disturbed microbial balance.[1-2] They conforms many health benefits upon consumption such as maintenance of normal cell growth and regeneration, normal stool consistency, healthy intestinal pH, normal bowel function, tone and condition, production of enzymes that aid in the digestion of lactose[2]. An understanding of the potential benefits of probiotic bacteria *Lactobacillus acidophilus* has led to the increasing use of the bacteria as dilatory adjunct. However most probiotic products have a short shelf life even when they are stored at low temperature.[3] Several studies have been shown that the number of viable bacteria in some of commercial product were actually below the desire level.[4-5]The desirable effect of bacteria can only be conferred if a sufficient number of bacteria are present at time of consumption. The objective of study is to evaluate the potential use of compression coating as a cell encapsulation method. Sodium alginate was chosen as the prime coating material because it is natural, safe and widely accepted in food, drink and pharmaceutical industries[6] This method is similar to tablet compression process, relatively simple easy to operate and do not use any liquid. The method has received renewed interest when applied in conjunction with gel forming polymers as coating material.

MATERIALS AND METHODS

Materials

Sodium alginate was kind gift of Alembic Pharmaceuticals Ltd. Baroda. HPMC-AS gifted by Signet chemical corporation, Mumbai. MRS broth (M369) & lyophilized powder of lactic acid bacteria strain (LAB) *Lactobacillus acidophilus* procured from High Media Labs Mumbai. All other chemicals and reagents used were of analytical grade.

Compression coating of probiotic cell containing powders

The freeze dried probiotic cell containing powders were compressed into pellets using S.S.
punches (diameter 6 mm flat surface) on rotary tablet press. The powders were compressed at a pressure of 60 MPa. The coating material used was a mixture of dry sodium alginate and HPMCAS in weight ratio of 9:1. The amount of coating used was 350mg. In coating process, half of the total amount of coating material was first used to fill 13mm die. The compressed cell pellet was carefully positioned on center of die before rest of the coating material was poured on top of it. The pressure employed was the same as that used to compress the freeze dried cell containing powder.

Content of viable cell counts

The survival of free and encapsulated cells in simulated gastric fluids (SGF) of pH 1.2, 2 and 4 was studied. The SGF was prepared according to USP XXII method [7]. All the tests were carried out according to USP 2 paddle method at 100 rpm, 37 ^{0}C with 600 ml of SGF [8]. The cell pellets were tested for contents of viable cell counts of probiotic lactobacilli using standard plate count (SPC) method [9]. The SPC (Standard plate count) test was performed after making the serial dilutions of the revived bacterial cultures [10]. The following scheme enlists the steps involved in the same.

RESULT AND DISCUSSION

Table 1: Viable cell count of L. *acidophilus* from pellets at SGF of pH

Time (min)	SGF (pH)	Plate No.	Dilution	No. of Colonies	Corresponding no. of microorganisms	Average cell count
30	1.2	8	10^8	50	5 x 10^4	Approx. 10^2
		9	10^9	7	7 x 10^3	
		10	10^{10}	2	2 x 10^1	
	2	8	10^8	150	15 x 10^9	Approx. 10^6
		9	10^9	11	11 x 10^6	
		10	10^{10}	7	7 x 10^4	
90	2	8	10^8	155	15 x 10^5	Approx. 10^4
		9	10^9	10	10 x 10^3	
		10	10^{10}	6	7 x 10^4	

Generally, there was exponential reduction in number of viable cells with decrease in pH of SGF with time for all cases. The results indicated that cells were very vulnerable when they were exposed to SGF of pH 2 and below. The numbers of viable cells were decreased dramatically from 10^6 to 10^2 CFU/mg. after they were exposed to SGF of pH 1.2 for 30 min.

The cells survived better above pH 2 where cell viability decreased from 10^6 to 10^4 CFU/mg. after 90 min. of exposure. The loss of viable cells was less severe at pH 4, only 10^1CFU/mg. loss found to be at pH 2.

The survival rate of encapsulated cells (coated with 350 mg of coating material) was significantly higher than that of free cells after 2h exposure to SGF of pH 1.2 and 2. Under test condition there were 10^4 to 10^5 fold increments in survival rate of encapsulated cells to that of free cells.

CONCLUSION

The results of experimental studies proved that the direct compression is one of the suitable methods for encapsulation. The encapsulated cells shown improved survival rate when exposed to acidic medium compared to the free cells. Sodium alginate is sodium salt of alginic acid. It is soluble at neutral pH and hydrates to form a viscous solution at this pH. However, it is insoluble at pH below 3 and swells considerably at this pH. Hence, the formation of hydrogel by the increasing concentration of polymer around the cell pellet was responsible for protection of probiotic bacterial cells. The probiotic cells could be released to the appropriate site in human gastro intestinal tract.

ACKNOWLEDGEMENT

The authors are thankful to Signet Chemical Corporation, Mumbai, for gift sample HPMC-AS (HF) Alembic Pharmaceuticals Ltd. Baroda for sample of Sodium alginate. Authors also wish to thanks to High Media Labs Mumbai for providing lactic acid bacteria strain powder.

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