IJPSR (2014), Vol. 5, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 November, 2013; received in revised form, 15 January, 2014; accepted, 09 March, 2014; published 01 May, 2014

EFFECT OF DIFFERENT EXCIPIENTS AND PACKAGING MATERIALS ON COMMERCIAL PREPARATION OF PROBIOTIC FORMULATION

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Keywords:

Probiotic, Formulation, API, Excipients, Packaging, Laminates, Shelf-life

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ABSTRACT: Probiotic formulations are now widely accepted throughout the world for therapeutic applications as these organisms have a long history of safe use (GRAS). There are two main challenges to make a probiotic formulation viable for commercial utilization. The first one is the quality of health attributes that the probiotic organisms can confer and the second one is the stability of the formulation till the end of its shelf life. With our promising probiotic blend (a mixture of probiotic organisms) as API (Active Pharmaceutical Ingredient), we have tried to develop a sachet containing probiotic formulation by using different types of excipients and packaging options. Among the excipients that were tried, a commercial variety of mannitol was found to be most suitable. Appropriate packaging material is most vital for enhancement of the shelf life of a formulation. Three types of laminates were used for making sachets for protecting the probiotic formulation against moisture and oxygen. Among them, type 1 (combination of Paper/ Polyethylene/Aluminium foil/ Polyethylene) was found to give maximum protection when stored at 25^{o}C temperature and 60 % RH.

INTRODUCTION: The probiotics are described as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. The Joint FAO/WHO Expert Panel concluded that probiotic strains should belong to two genera, *Lactobacillus* and *Bifidobacterium* which must survive the passage through the digestive tract and can proliferate in the large intestine. *Lactobacillus* species from which probiotic strains have been recommended include *L. Acidophilus, L. johnsonii, L. casei,L. rhamnosus, L. gasseri* and *L. reuteri*.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(5).1830-36

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(5).1830-36

Bifidobacterium strains include *B. bifidum, B. longum, B. lactis, B. infantis.* However, *Saccharomyces boulardii*, *Escherichia coli* and enterococcus strains can also be used as probiotics.

These organisms have a historical track record of safety (Generally Recognized as Safe, GRAS). Because of their widespread acceptance and general lack of side effects, probiotics are getting increasingly popular throughout the world and consequently are now used widely for various clinical indications. In commercial aspect, probiotics have some basic requirements for the development of marketable products. First of all, probiotic bacteria in a particular product must be present in sufficient numbers ranging from 10^6 to 10^9 colony forming units/dose. Their physical and genetic stability during storage must be guaranteed and all their properties must be maintained throughout the shelf life of the product.

Apart from active pharmaceutical ingredients, two other important factors that play a major role are the excipients that are blended with API and the packaging that protects the formulation as a whole. Keeping water activity (a_w) and product temperature low and minimizing exposure to oxygen, enhance the stability of probiotic formulations. Probiotic products are naturally hygroscopic and tend to absorb any available moisture. Therefore, for optimal packaging of probiotics high moisture/oxygen barrier materials such as glass or aluminium is recommended. The barrier performance of different types of packaging can be compared by Moisture Vapour Transmission Rate (MVTR) and Oxygen Transmission Rate (OTR). MVTR and OTR are the steady state rate at which water vapour and oxygen respectively permeate through a film at specified conditions of temperature and relative humidity.

Mannitol is widely used in pharmaceutical formulations and which can also be used as diluent (10-90%). The use of mannitol as excipients usually has some advantages. Mannitol has low glycemic index. So it does not increase the blood glucose level. Mannitol is also very less hygroscopic and may thus be used with moisture-sensitive active ingredients [2]. Additionally the pleasant taste and mouthfeel of mannitol makes it a popular choice as an excipient for pharmaceutical formulations with probiotic.

In our present study, we have prepared a probiotic formulation in sachet using *Lactobacillus acidophilus* and *Bifidobacterium lactis*. Different types of excipients and packaging materials were used to examine their role in the shelf-life of the said formulation.

MATERIALS AND METHODS:

Determination of Viable Count (CFU/g) of Samples: 1g of API or formulation was taken into 99ml of MRD (Maximum Recovery Diluent); composed of peptone 1g/L, NaCl 8.5g/L, pH: 7 ± 0.2 at 25 ºC (after autoclaving). Then the suspension was further appropriately diluted by decimal dilution method so that the theoretical colony forming unit of the dilution was between 40-240 colonies per plate. Aliquot from 1ml from the desired dilution was pour plated using MRS [3] (HIMEDIA, India) agar media. The plates were then incubated at 37ºC for 72 hours anaerobically (Anaerobic Gas Jar, HIMEDIA). The total count in the sample was determined by counting total colonies on plates and the result were expressed in CFU/g.

Determination of Water Activity (a_w) of Samples: The water activity of the samples was determined by using Rotronic Hygrolab 3 in humidity (22% RH) and temperature (25^0C) controlled condition. Samples were taken in a specific sample container and immediately placed in measuring chamber. The water activity of the sample was displayed in 5 minutes or less using a_w Quick mode (i.e. accelerated water activity analysis). The a_w reading along with the temperature was recorded.

Preparation of Sachet: The powdered probiotic formulation was granular in nature and had very good flow characteristics itself. 1g of the formulation was the minimum pack amount in a sachet containing the requisite dose. The primary mode of packaging was selected in laminated sachet. Critical function of flexible laminated packaging is to keep the product inside the sachet away from any ingress or outgress of moisture and oxygen. General regression models were used to predict the moisture and oxygen barrier properties of various packaging options in different atmospheric conditions. Three packaging options were selected for protection of probiotic formulation. The laminates were as follows (outer to inner layer) **(Figure 1)**

1. **Type 1:** Paper/ Polyethylene (PE)/ Aluminium foil/ Polyethylene (PE)
2. **Type 2:** Polyethylene terephthalate (PET)/ Aluminium foil/ Polyethylene(PE)
3. **Type 3:** Polyethylene terephthalate (PET)/ Aluminium foil/ Sealant coating with Vinyl Malic Copolymer of Hydrocarbon (VMCH)

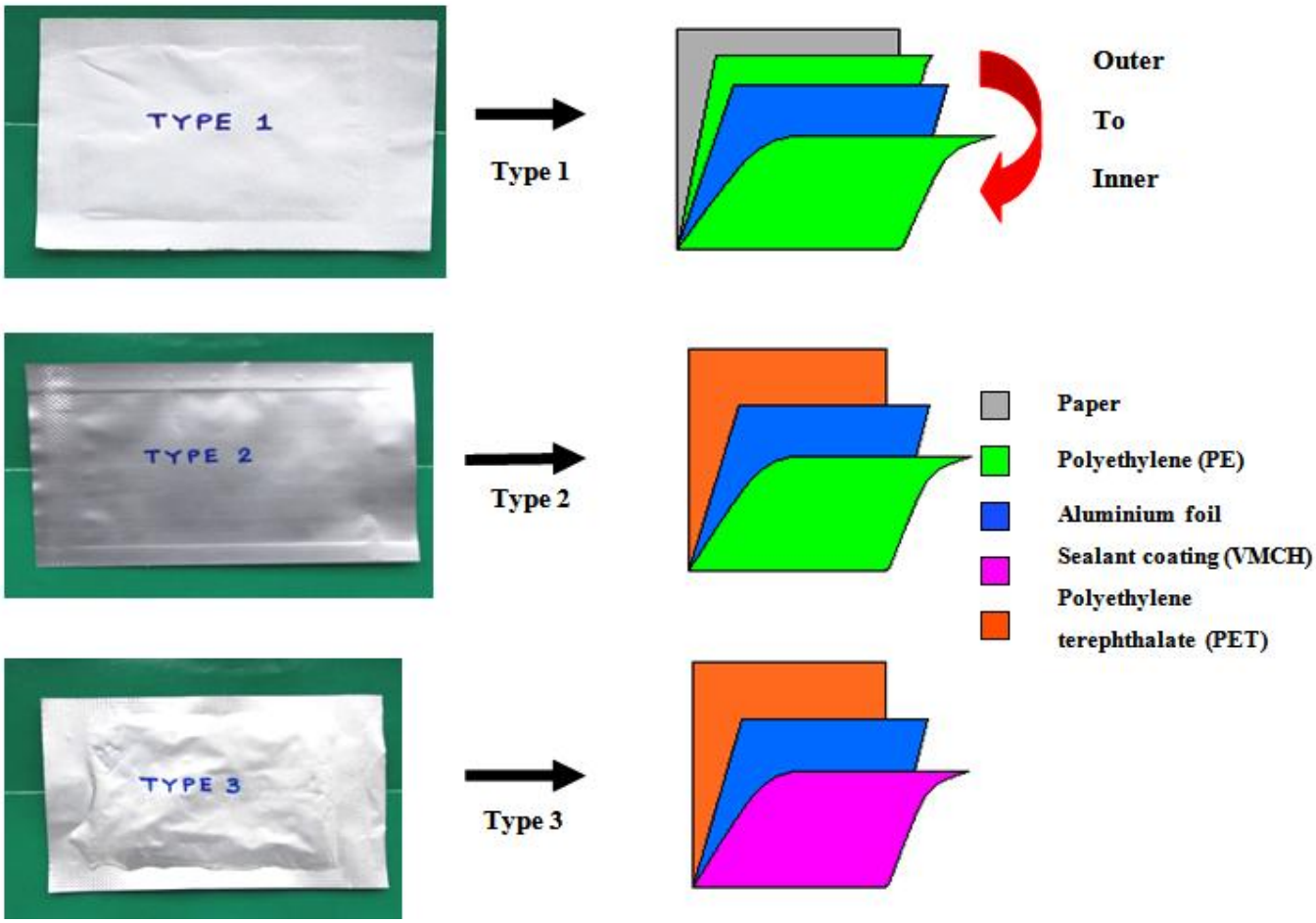


FIGURE 1: SACHETS WITH THEIR RESPECTIVE SCHEMATIC CROSS-SECTIONAL VIEW OF LAMINATES

Preparation of Excipients:

Mannitol Granules: Wet Mannitol granules were prepared by mixing mannitol with 15% (w/v) polyvinylpyrrolidone (PVP) and were passed through mesh (size 16). The wet granules were then dried at 70 ºC till water activity reached ≤0.2 (25^{0}C). Finally the dry granules were passed through mesh (size 24).

Dibasic Calcium Phosphate (DCP) Granules: Wet DCP granules were prepared by mixing DCP with 15% (w/v) polyvinylpyrrolidone (PVP) and were passed through mesh (size 16). The wet granules were then dried at 70ºC till water activity reached ≤0.2 (25^{o}C). Finally the dry granules were passed through mesh (size 24).

Filling of Sachet: The API, Mannitol granules/ Dibasic Calcium Phosphate granules/ Pearlitol DC (a commercial variety of mannitol granules, Roquette) and flavor (0.2%) were mixed and sieved through a mesh (size 24) for ensuring homogeneous mixture. Total mixture was divided into three aliquots for three types of sachets. Approximately 1.1g of formulation was put into each sachet and heat sealed immediately. The whole operation was carried out in humidity and temperature controlled environment (25% RH, 25ºC). The sachets were then put into a stability chamber maintaining 25ºC and 60% RH. The stability study was carried out following ICH guidelines.

RESULT AND DISCUSSION: Due to the universal acceptance and general lack of side effects, probiotics are getting increasingly popular among the community and consequently are now used widely for various therapeutic purposes. The prospect of the probiotic market is expanding in a steadfast way which looks bright. But at the same time, there are formidable challenges that are to be encountered to make the product stable through out its shelf life.

The factors like moisture, oxygen toxicity, temperature and excipient selection mainly play key roles for maintaining stability of the probiotic formulations.

In our present study, stability of probiotic formulations was carried out in three different sachets. For each type of sachets three types of excipients were used for testing the compatibility with API. The sachets were kept in the stability chamber maintaining 25ºC and 60% RH. Stability study data upto the 6th month has been presented here **(Figure 2, 3, 4)**.

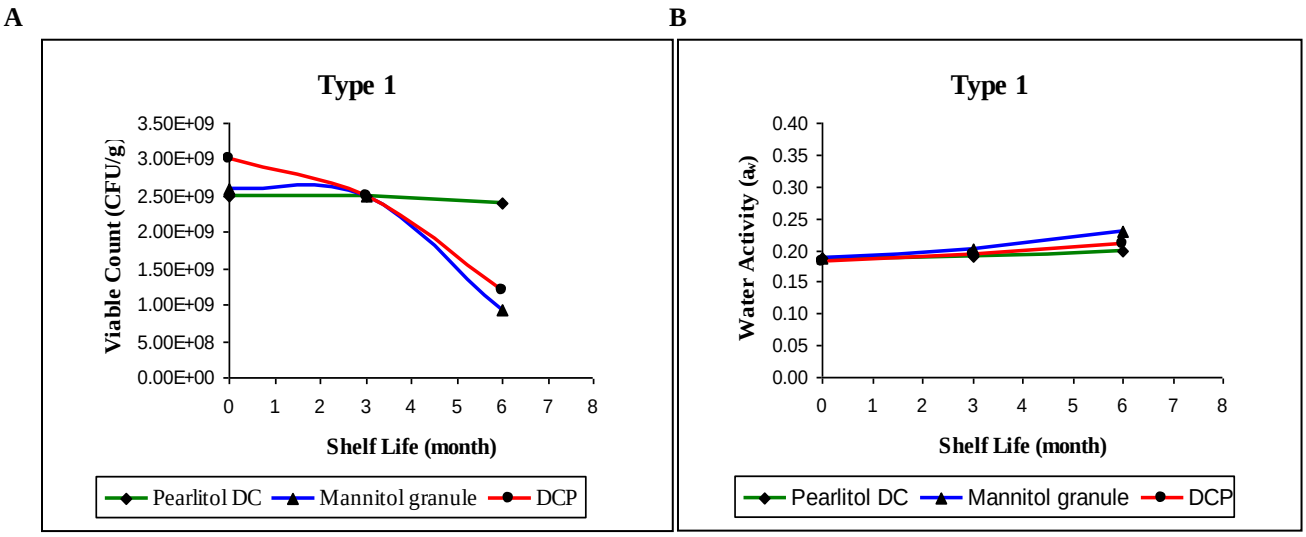


FIGURE 2: EFFECT OF PACKAGING (TYPE 1) ON VIABILITY AND WATER ACTIVITY OF PROBIOTIC FORMULATIONS

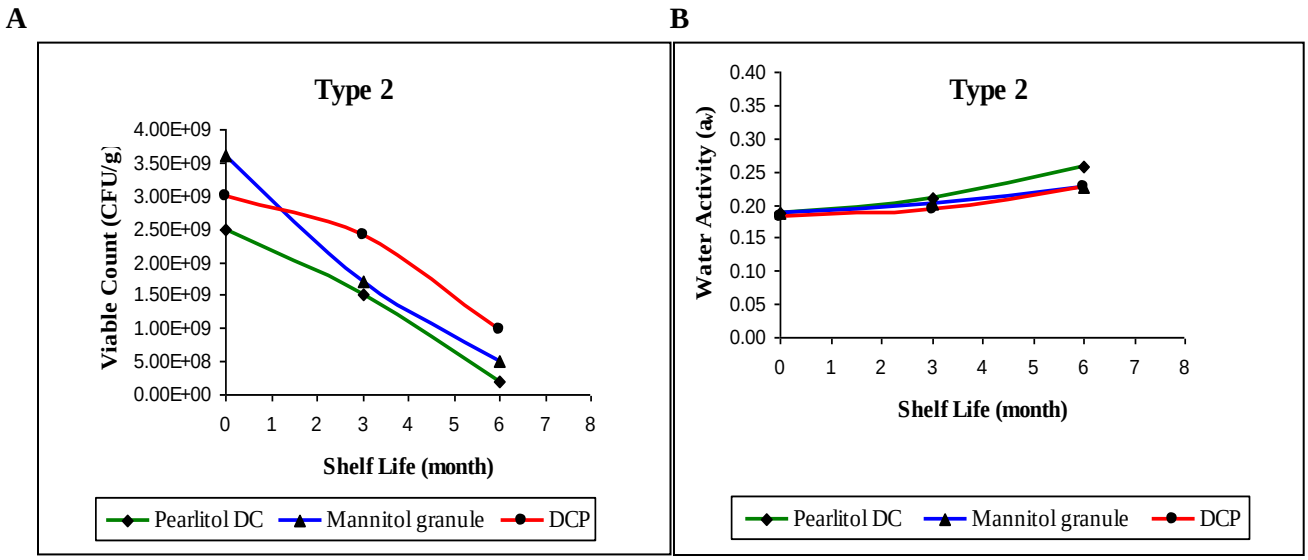


FIGURE 3: EFFECT OF PACKAGING (TYPE 2) ON VIABILITY AND WATER ACTIVITY OF PROBIOTIC FORMULATIONS

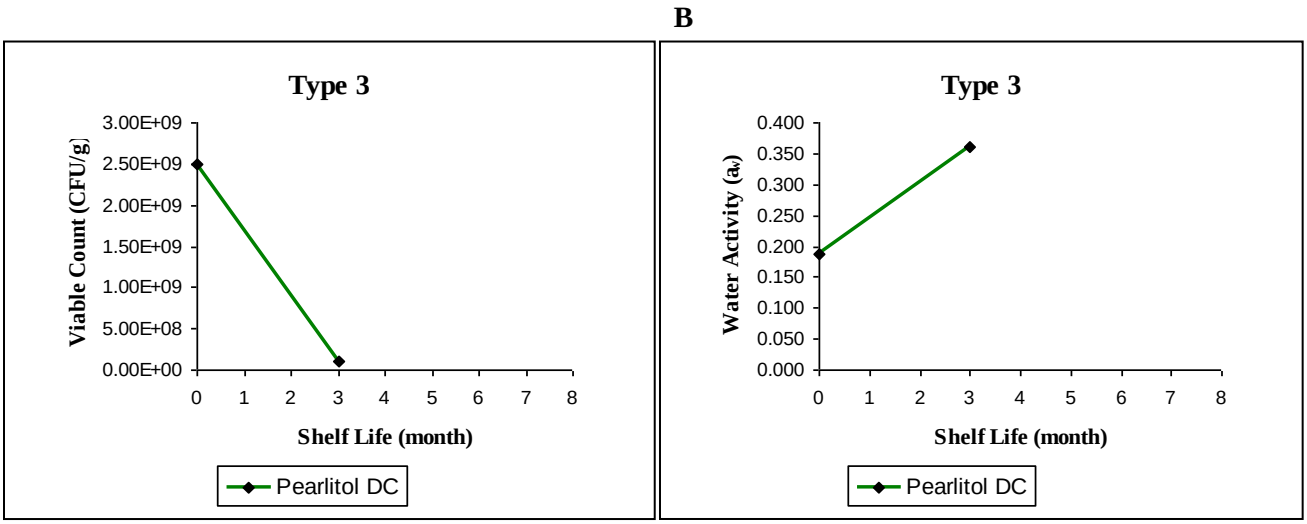


FIGURE 4: EFFECT OF PACKAGING (TYPE 3) ON VIABILITY AND WATER ACTIVITY OF PROBIOTIC FORMULATIONS

The excipients are generally pharmacologically inactive substances which are used as carrier for the active ingredients of a formulation. Mannitol is widely used in pharmacological formulations. It has the property of excellent stability, low hygroscopicity, good solubility in different solvents, low caloric values and that is why it is reported as a better excipient for active ingredients which are moisture sensitive [2, 4]. It was found in our experiments; type 1 sachet containing Pearlitol DC was the most suitable excipient. Both water activity (a_w) and viable count (CFU) were stable upto the 6^{th} month **(Figure 2)**. But in case of DCP and mannitol granule water activity increased which got reflected in decreased viable counts (CFU/g) with due course of time.

The type 2 sachet also reflects the same trend of results **(Figure 3)**. In case of type 3 sachet where only Pearlitol DC was used as excipient, it was found that after three months both the viable count (CFU/g) and water activity (a_w) were deteriorated **(Figure 4)**. The Pearlitol DC was found to be the best excipient in comparison with prepared mannitol granules and DCP granules in terms of viable count (CFU/g) and water activity (a_w) of the formulation when stored in the stability chamber (25^0C temperature, 60% RH). Incorporation of mannitol preserves secondary protein conformation and improve stability of cell envelopes of probiotic bacteria during storage at room temperature at low a_w s [5].

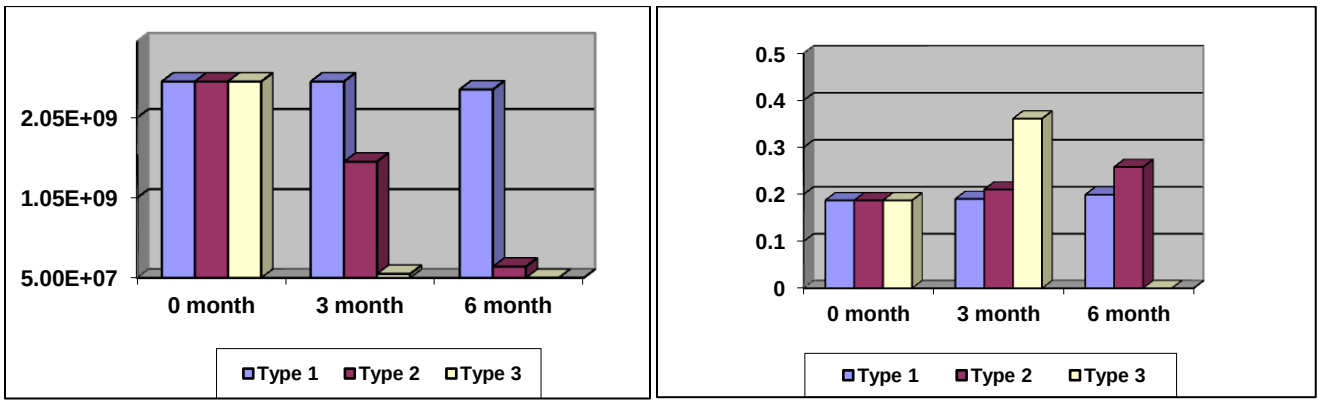
The mannitol granule (DC grade) also imparts good flowability [6]. It was reported [7] that the excipients like lactose, ascorbic acid and inulin have a positive impact on the shelf life of formulation using probiotic organism *Pediococcus sp.* Mannitol also contribute in the protection of probiotic bacteria during drying and storage at room temperature [8]. Bora *et al*, [9] revealed the importance of excipients in making successful formulation with *Bacillus coagulans* spores.

The microorganisms usually are associated with three types of water viz. free water, bound water and preserved water [9]. The free water content is found to be the most deleterious to the stability of probiotic microorganisms [9] and this can be measured by water activity. Keeping water activity of the probiotic formulation low ($\leq$ 0.2) helps to maintain the shelf life of the formulation.

The role of appropriate packaging is most vital in order to maintain optimum water activity of any probiotic formulation. It also helps to enhance the shelf life of the product. The selection of packaging material for protection of the formulation in terms of barrier function is the most critical parameter for packaging development.

The barrier function helps to control the moisture vapour transmission rate and oxygen transmission rate, thus maintaining the water activity of the formulation [10].

Glass, a chemically inert, non-porous, rigid packaging material can give almost 100% barrier against moisture and oxygen transmission. Among the flexible packaging, aluminium foil of different thickness gives protection against moisture and oxygen as compared to glass. Moisture barrier properties of extrusion-coated polymers also play an important role. The general selection of moisture barrier polymers includes low and high density polyethylene (LDPE-HDPE), polypropylene (PP), and polyethylene terephthalate. These also include cyclo-olefine copolymers, metallocene, nanocomposites etc. Considering all subsequent data of water activity and viable count for all three types of sachet, type I was found to be the most suitable. More specifically when Pearlitol DC was used as excipient for making probiotic formulation using type I sachet, the water activity and viable count were found most stable Y axis should have legend: CFU/g **(Figure 5)**.



A. CFU/g: Viable Counts **B.** Y axis should have legend : a_w: Water Activity

FIGURE 5: COMPARATIVE STABILITY STUDY OF PROBIOTIC FORMULATION USING PEARLITOL DC AS EXCIPIENTS IN TYPE 1, TYPE 2, TYPE 3

Here, we have used three types of multilayered laminated sachets of different material combinations. All sachets have aluminium foil as one of the layer of varying thickness to optimize the barrier protection along with some polymer or copolymer layers and polymer coated paper. The samples were measured in specified atmospheric condition (temperature and humidity) in order to achieve enough variable for regression analysis. Another factor which is important for satisfactory barrier package is seal integrity. Poor quality of seals can negate a laminate's good barrier by allowing vapour transmission through channel leakers. So proper seal strength and fast sealing techniques using high speed machine are also very important criteria of good laminates which can be achieved by correct choice of different thickness of the polymer layers.

CONCLUSION: In our present work it was found that excipient pearlitol DC and type 1 sachet is the best combination to provide desired shelf life to the probiotic product.

ACKNOWLEDGEMENT: Authors are thankful to the management of East India Pharmaceutical Works Limited, Kolkata for providing facilities and encouragement.

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How to cite this article:
Das S., Bhattacharjee D., Mannac A., Basu S., Chowdhury S., Mukherjee S., Bhattacharyya B.K.: Effect of different excipients and packaging materials on commercial preparation of probiotic formulation. *Int J Pharm Sci Res* 2014; 5(5): 1830-36.doi: 10.13040/IJPSR.0975-8232.5 (5).1830-36.