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DEVELOPMENT AND STABILITY STUDY OF PROBIOTIC CAPSULES

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ABSTRACT

Robotics are live microorganisms that provide health benefits to the host when administered in adequate amounts. The growing interest in gut health and preventive healthcare has increased the demand for stable and effective probiotic dosage forms. However, maintaining the viability of probiotic microorganisms during manufacturing, storage, and gastrointestinal transit remains a significant challenge. The present study focuses on the development and stability evaluation of a probiotic capsule formulation containing selected probiotic strains.

The probiotic capsules were formulated using suitable probiotic cultures along with compatible excipients to ensure adequate flow properties, content uniformity, and microbial viability. The formulation process was optimized to minimize environmental stress and preserve the viability of the probiotic organisms. The prepared capsules were evaluated for various pharmaceutical parameters, including appearance, weight variation, moisture content, disintegration time, and viable cell count.

Stability studies were conducted according to standard guidelines under different storage conditions to assess the effect of temperature and humidity on the viability and quality of the probiotic capsules. Samples were analyzed at predetermined intervals for physical characteristics, moisture content, and colony-forming units (CFU). The results demonstrated that the developed probiotic capsules maintained acceptable physicochemical properties and retained sufficient viable cell counts throughout the study period when stored under recommended conditions.

The findings indicate that the developed probiotic capsule formulation is stable, effective, and suitable for delivering viable probiotic microorganisms. This study highlights the importance of proper formulation strategies and storage conditions in ensuring the quality and therapeutic efficacy of probiotic products. The developed formulation may serve as a promising approach for enhancing probiotic stability and consumer acceptance.

KEYWORDS: ***Robotics, Probiotic Capsules, Stability Studies, Viability, Colony Forming Units (CFU), Lactobacillus, Bifidobacterium, Pharmaceutical Formulation***.

1. INTRODUCTION

The association of Robotics with well-being has a long history. More than a century has passed since Tissier observed that gut microbiota from healthy breast-fed infants were dominated by rods with a bifid shape (bifidobacteria), which were absent from formula-fed infants suffering from diarrhoea, establishing the concept that they played a role in maintaining health. Since then, a series of studies have supported this association, but they were originally poorly designed and controlled and faced practical challenges such as strain specificity of properties and the slow growth of probiotics in substrates other than human milk. Over time, they have successfully evolved, with the more recent ones accumulating more substantial evidence that probiotic bacteria can contribute to human health. These data have coincided with the increasing consumer awareness about the relationship between health and nutrition, creating a supporting environment for the development of the functional food concept introduced to describe foods or food ingredients exhibiting beneficial effects on the consumers' health beyond their nutritive value. The functional food market is expanding, especially in Japan—its birthplace—with further growth prospects in Europe and the United States, and in most countries, the largest share of its products is held by Robotics [1, 2]. The reported beneficial effects of probiotic consumption include improvement of intestinal health, amelioration of symptoms of lactose intolerance, and reduction of the risk of various other diseases, and several well-characterized strains of Lactobacilli and Bifidobacteria are available for human use [3, 4]. Nevertheless, despite the promising evidence, the role of probiotics in human health as well as the safety of their application should be further investigated, as the current knowledge of the characteristics that are necessary for their functionality in the gut is not complete.

DEFINITION OF PROBIOTICS AND OTHER RELATED TERMS

Etymologically, the term probiotic is derived from the Greek language meaning “for life,” but the definition of probiotics has evolved, simultaneously with the increasing interest in the use of viable bacterial supplements and in relation

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to the progress made in understanding their mechanisms of action. The term was originally used to describe substances produced by one microorganism that stimulated the growth of others and was later used to describe tissue extracts that stimulated microbial growth and animal feed supplements exerting a beneficial effect on animals by contributing to their intestinal flora balance [5]. Until recently, the most widely used definition, which contributed to the development of the probiotic concept in several ways, was that of Fuller: "probiotics are live microbial feed supplements which beneficially affect the host animal by improving microbial balance" [6]. The definition used at present was given by the Food and Agriculture Organization of the United Nations and the World Health Organization, according to which probiotics are redefined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." In relation to food, the definition can be adjusted by emphasizing that the beneficial effect is exerted by the microorganisms "when consumed in adequate amounts as part of food" [7].

The term prebiotics was introduced by Gibson and Roberfroid in 1995 to describe food supplements that are nondigestible by the host but are able to exert beneficial effects by selective stimulation of growth or activity of microorganisms that are present in the intestine. Prebiotic substances are not hydrolysed nor absorbed in the gastrointestinal tract but are available as substrates for probiotics, and the most commonly used ones at present are non-digestible fructooligosaccharides. For practical reasons, the combination of probiotics and prebiotics has been described as conbiotics by certain authors and as symbiotics by others [8, 9]. Although prebiotics seem to have a role in health promotion, this needs to be supported by further studies. During the last few years, the concept of functional food has also been developed in order to describe foods containing ingredients with positive effects on host health beyond their nutritive value. They include those products that contain biologically active components that improve health, such as probiotics [1].

MICROBIAL SPECIES WITH APPLICATIONS AS PROBIOTICS

Taking into consideration their definition, the number of microbial species that may exert probiotic properties is impressive. Some of the most important representatives are listed in Table 1. As far as nutrition is concerned, only the strains classified as lactic acid bacteria are of significance, and among them, the ones with the most important properties in an applied context are those belonging to the genera Lactococcus and Bifidobacterium [10]. Lactic acid bacteria are Gram-positive, catalase-negative bacterial species able to produce lactic acid as the main end-product of carbohydrate fermentation. The genus Bifidobacterium is therefore rather traditionally than phylogenetically listed among them as they use a separate metabolic pathway. Two other species playing an important role in the food industry, particularly dairy products, although not strictly considered as probiotics, are Streptococcus thermophilus and Lactococcus lactis, two of the most commercially important lactic acid bacteria [11].

1 . Mainly used for animals.
2 . Recently reclassified as B. animalis subsp. lactis [59].
3 . Little is known about probiotic properties.

It is important to mention that since probiotic activities are strain-related, strain identification is recommended in order to establish their suitability and performance for industrial application. This is achieved by a combination of phenotypic tests followed by genetic identification using molecular techniques like DNA/DNA hybridisation, 16S RNA sequencing, and so forth [7].

DESIRABLE PROBIOTIC PROPERTIES

For a potential probiotic strain to be able to exert its beneficial effects, it is expected to exhibit certain desirable properties. The ones currently determined by in vitro tests are acid and bile tolerance, which seems to be crucial for oral administration, adhesion to mucosal and epithelial surfaces, an important property for successful immune modulation, competitive exclusion of pathogens, as well as prevention of pathogen adhesion and colonisation, antimicrobial activity against pathogenic bacteria, Bile salt hydrolase activity.

Nevertheless, the value of these parameters is still under debate as there are matters of relevance, in vivo and in vitro discrepancies, and a lack of standardization of operating procedures to be considered. As there are no specific parameters essential to all probiotic applications, the best approach to establish a strain's As far as the final product is concerned, the probiotic dose levels should be based on the ones found to be efficacious in human studies, and the colony-forming units per gram of product is an important parameter. Although the information about the minimum effective concentrations is still insufficient, it is generally accepted that probiotic products should have a minimum concentration of 106 CFU/mL or gram and that a total of some 108 to 109 probiotic microorganisms should be consumed daily for the probiotic effect to be transferred to the consumer. Furthermore, the properties are target population and target physiologic function-specific studies [12–14].

Strains must be able to grow under manufacturing and commercial conditions and should retain viability under normal storage conditions [3, 15]. Viability is, by definition, a prerequisite for probiotic functionality as it potentiates mechanisms such as adherence, reduction of gut inflammation, and immunomodulation and constitutes an industrial challenge [16, 17]. Nevertheless, certain studies have demonstrated that viability is not necessary for all probiotic effects, as not all mechanisms or clinical benefits are directly related to viability, and that even cell wall components on some probiotic bacteria or probiotic DNA may have significant health effects. Thus, for certain probiotic strains, optimal growth during the initial production steps might be sufficient, and they may not need to retain good viability during storage [18, 19].

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MECHANISMS OF PROBIOTIC ACTIVITY

Robotics has various mechanisms of action, although the exact manner in which they exert their effects is still not fully elucidated. These range from bacteriocin and short-chain fatty acid production, lowering of gut pH, and nutrient competition to stimulation of mucosal barrier function and immunomodulation. The latter in particular has been the subject of numerous studies, and there is considerable evidence that probiotics influence several aspects of the acquired and innate immune response by inducing phagocytosis and IgA secretion, modifying T-cell responses, enhancing Th1 responses, and attenuating Th2 responses [20–22].

PROBIOTICS AND FOOD PRODUCTS

The range of food products containing probiotic strains is wide and still growing. The main products existing in the market are dairy-based ones, including fermented milks, cheese, ice cream, buttermilk, milk powder, and yogurts, the latter accounting for the largest share of sales [23, 24].

Nondairy food applications include soy-based products, nutrition bars, cereals, and a variety of juices as appropriate means of probiotic delivery to the consumer [25, 26]. The factors that must be addressed in evaluating the effectiveness of the incorporation of the probiotic strains into such products are, besides safety, the compatibility of the product with the microorganism and the maintenance of its viability through food processing, packaging, and storage conditions. The product's pH, for instance, is a significant factor determining the incorporated probiotic's survival and growth, and this is one of the reasons why soft cheeses seem to have several advantages over yoghurt as delivery systems for viable probiotics to the gastrointestinal tract [27–29]. Current technological innovations provide ways to overcome probiotic stability and viability issues, offering new options for their incorporation into new media and the subsequent satisfaction of increasing consumer demand. Microencapsulation technologies have been developed to protect the bacteria from damage caused by the external environment. By the introduction of a straw delivery system containing a dry form of the probiotic bacterium, beverage manufacturers can now provide it to the consumer. In addition, viable spores of a spore-forming probiotic are available in the market, offering advantages during processing. At the same time, the potential of lantibiotics'—substances with antimicrobial properties—production by bifidobacteria is being explored in order to be applied in the food area [30, 31].

ANTIBIOTIC-ASSOCIATED DIARRHOEA

Mild or severe episodes of diarrhoea are common side effects of antibiotic therapy, as the normal microflora tends to be suppressed, encouraging the overgrowth of opportunistic or pathogenic strains. The spectrum may range from diarrhoea without mucosal abnormality to pseudomembranous colitis. The latter is a severe form of antibiotic-associated diarrhoea (caused by Clostridium difficile, cytotoxic strains of which may emerge after antibiotic use). The name of the condition is derived from the plaque-like adhesion of fibrinopurulent material to the damaged mucosal layer, and it is characterized by diarrhoea, abdominal distention, vomiting, fever, and leukocytosis, and if untreated, might lead to complications such as toxic megacolon and perforation. Treatment consists of withdrawal of the causal antibiotic agent, correction of the electrolyte disorders, and, in severe cases, therapy with metronidazole or vancomycin. Treatment with probiotics has been used in clinical practice, with L. rhamnosus and S. boulardii being administered. Several studies that have been carried out suggest that probiotic use is associated with a reduced risk of antibiotic-associated diarrhoea [32, 33]. A recent meta-analysis evaluating the available evidence on probiotics for the prevention and treatment of antibiotic-associated diarrhoea concluded that probiotic administration (namely, L. rhamnosus, L. casei, and the yeast S. boulardii, as these are the probiotics predominantly included in the majority of trials) is associated with a reduced risk of the condition. Matters for future research include the optimal dose of the probiotic preparation and the comparative effectiveness of different probiotic interventions [34].

INFECTIOUS DIARRHOEA

Treatment and prevention of infectious diarrhoea are probably the most widely accepted health benefits of probiotic microorganisms. Rotavirus is the most common cause of acute infantile diarrhoea in the world and a significant cause of infant mortality. The virus replicates in the highly differentiated absorptive columnar cells of the small intestinal epithelium, and the normal microflora seems to play an important role in the host response to the infection, as it has been shown that absorption of antigens is more enhanced in germ-free than in normal mice [35]. Probiotic supplementation of infant formulas has been aimed both at the prevention of rotaviral infections and the treatment of established disease. Well-controlled clinical studies have shown that probiotics such as L. rhamnosus GG, L. reuteri, L. casei Shirota, and B. animalis Bb12 can shorten the duration of acute rotavirus diarrhoea, with the strongest evidence pointing to the effectiveness of L. rhamnosus GG and B. animalis Bb12 [36–38]. The proposed mechanisms include competitive blockage of receptor site signals regulating secretory and motility defences, enhancement of the immune response, and production of substances that directly inactivate the viral particles. In addition to rotavirus infection, there is evidence that certain foods as well as non-food probiotic strains can inhibit the growth and adhesion of a range of diarrhoeal syndromes. The benefit of probiotics such as L. reuteri, L. rhamnosus GG, L. casei, and S. boulardii in reducing the duration of acute diarrhoea in children has been demonstrated [37, 39]. For example, in a prospective, randomized, controlled French study conducted among children in day care, the administered probiotic yoghurt product containing L. casei shortened the mean duration of diarrhoea significantly compared to the conventional one [40]. Several studies have investigated the efficacy of probiotics in the prevention of travellers' diarrhoea in adults. Although results are quite contradictory, due to differences in study populations, type of probiotic being investigated, applied doses, as well as trip

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destination and traveller compliance, L. rhamnosus GG, S. boulardii, L. acidophilus, and B. bifidum seem to exhibit significant efficacy [41–43]. Furthermore, numerous animal studies have indicated an inhibitory effect of probiotics against enteropathogens mainly through the production of bacteriocins [44].

LACTOSE INTOLERANCE

Lactose intolerance is a genetically determined beta-galactosidase deficiency resulting in the inability to hydrolyse lactose into the monosaccharides glucose and galactose. Upon reaching the large bowel, the undigested lactose is degraded by bacterial enzymes, leading to osmotic diarrhoea. Acquired, usually reversible, causes of beta-galactosidase deficiency include pelvic radiotherapy, which damages the mucosa, as well as infection with rotavirus, which infects lactase-producing cells, and short bowel syndrome. Lactose-intolerant individuals develop diarrhoea, abdominal discomfort, and flatulence after consumption of milk or milk products. Although conventional yoghurt preparations, using S. thermophilus and L. delbrueckii ssp. Bulgaricus is even more effective in this direction, partly because of higher beta-galactosidase activity. Improvement of lactose metabolism is a claimed health benefit attributed to probiotics and seems to involve certain strains more than others and in specific concentrations. Therefore, and as certain individuals have responded positively to probiotic supplementation, clinicians should consider it as a therapeutic alternative [45, 46].

PROBIOTICS AND ALLERGY

Recent evidence suggests that exposure to bacteria in early life may exhibit a protective role against allergy, and in this context, probiotics may provide a safe alternative microbial stimulation needed for the developing immune system in infants. At the same time, they improve mucosal barrier function, a property that is considered to contribute to moderating allergic response. The role of intestinal microbiota in allergy is supported by observations of their quantitative as well as qualitative differences among children and infants suffering from allergies and healthy ones, the former exhibiting colonization by a more adult-like type of microflora [42, 47–49]. These probiotic effects seem to particularly involve food allergy and atopic dermatitis. The latter is a common chronic relapsing skin disorder of infancy and childhood, with hereditary predisposition being an important component of its pathogenesis, together with the individual's exposure to environmental allergens. A limited number of strains have been tested for their efficacy in the treatment and prevention of allergy in infants. In a recent study of breast-fed infants suffering from atopic eczema, B. lactis and L. rhamnosus GG were found to be effective in decreasing the eczema severity. Furthermore, L. rhamnosus GG has been found successful in preventing the occurrence of atopic eczema in high-risk infants, when supplied prenatally to selected mothers who had at least one first-degree relative with atopic eczema, allergic rhinitis, or asthma [50]. Robotics, however, has not been very successful in alleviating symptoms of asthma [51]. As far as food allergy is concerned, it is described as an immunologically mediated adverse reaction against dietary antigens leading to secondary intestinal inflammation and disturbances. The mechanisms of the immune modulating effect of L. rhamnosus GG are not entirely understood but seem to be related to the antigen's transport across the intestinal mucosa [52]. Recently, the use of probiotic preparations in adults with milk hypersensitivity—not lactose intolerance—has been studied, concluding that certain strains may suppress the milk-induced inflammatory response and improve allergy symptoms; nevertheless, further study in the field is necessary [53, 54].

12. Other Health Benefits

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The list of health benefits mediated by probiotics is not limited to the ones mentioned so far and includes a range of promising effects that require further human studies to be substantiated. There is evidence that probiotic bacteria are dietary components that may play a role in decreasing cancer incidence. The exact mechanisms are under investigation, but studies have demonstrated that certain members of Lactobacillus and Bifidobacterium spp. decrease the levels of carcinogenetic enzymes produced by colonic flora through normalization of intestinal permeability and microflora balance, as well as production of antimutagenic organic acids and enhancement of the host's immune system [55, 56]. Furthermore, evidence suggests that food products containing probiotic bacteria could contribute to coronary heart disease prevention by reducing serum cholesterol levels as well as to blood pressure control. Proposed mechanisms include interference with cholesterol absorption from the gut, direct cholesterol assimilation, and production of end fermentation products that affect the systemic levels of blood lipids and mediate an antihypertensive effect. Nevertheless, these probiotic effects are still a matter of debate as further research is needed in long-term human studies [57]. Last but not least, probiotic strains administered in dairy products have been shown to improve the therapeutic outcome in women with bacterial vaginosis, most probably by supporting the normal vaginal lactobacilli microbiota [58].

2. LITERATURE REVIEW

- Sanders et al. (2019): Worked on the role of probiotics and prebiotics in intestinal health and disease. The authors concluded that probiotic microorganisms help maintain gut microbial balance, improve intestinal barrier function, and enhance immune responses. They emphasized that the health benefits of probiotics are strain-specific and depend on adequate dosage and viability.
- Hill et al. (2014): Reviewed the scientific definition and clinical applications of probiotics. The study concluded that probiotics are live microorganisms that confer health benefits when administered in adequate amounts. The authors highlighted the importance of selecting well-characterized strains with proven clinical efficacy.
- Anne et al. (2022): Worked on the prophylactic and therapeutic applications of probiotics in human health. The review concluded that probiotics are effective in preventing gastrointestinal disorders, improving immunity, and reducing the incidence of infectious diseases. They also reported beneficial effects in metabolic and inflammatory conditions.
- Liu et al. (2024): Studied the effects of probiotics on gut microbiota and host health. The authors concluded that probiotics positively influence microbial diversity, enhance the growth of beneficial bacteria, and improve intestinal homeostasis. Regular probiotic consumption was found to support digestive and immune health.
- Acosta-Rodríguez-Bueno et al. (2022): Worked on the clinical applications of *Bacillus clausii* as a probiotic. The study concluded that *Bacillus clausii* exhibits excellent stability, survives harsh gastrointestinal conditions, and is effective in the management of diarrhea and intestinal dysbiosis.
- Yadav et al. (2022): Reviewed the role of probiotics, prebiotics, and synbiotics as next-generation therapeutics. The authors concluded that combining probiotics with prebiotics improves microbial survival, colonization, and overall therapeutic effectiveness. Synbiotics were found to provide enhanced health benefits compared to probiotics alone.
- Zhang et al. (2023): Investigated the mechanisms through which probiotics influence gut microbiota and metabolism. The review concluded that probiotics produce beneficial metabolites, regulate host metabolism, and strengthen intestinal barrier integrity. These mechanisms contribute significantly to overall health promotion.
- Filipski et al. (2026): Worked on probiotics in gut health and immunity. The study concluded that probiotic supplementation improves immune function by stimulating beneficial immune responses and reducing inflammation. The authors emphasized their role in maintaining gastrointestinal health and preventing infections.
- Bulut et al. (2025): Reviewed the emerging concept of pharmabiotics and their applications in disease prevention and management. The study concluded that probiotics can serve as safe and effective adjuncts in the treatment of gastrointestinal, metabolic, and inflammatory disorders, offering promising alternatives to conventional therapies.
- Cho et al. (2025): Studied recent advances in therapeutic probiotics and their clinical applications. The authors concluded that probiotics have significant potential in personalized medicine due to their ability to modulate gut microbiota and immune responses. They also highlighted the need for improved formulation strategies to ensure probiotic stability and viability.

3. MATERIALS REQUIRED

1. ACTIVE PHARMACEUTICAL INGREDIENTS

CURD

Curd is a fermented dairy product prepared by adding beneficial lactic acid bacteria to milk. During fermentation, these bacteria convert lactose (milk sugar) into lactic acid, which causes the milk to coagulate and form a thick, creamy texture. Curd is rich in proteins, calcium, vitamins, and probiotics that help maintain gut health and improve digestion. It is widely consumed as a nutritious food and is commonly used in the preparation of probiotic formulations due to the presence of beneficial microorganisms such as Lactobacillus species. Curd has a slightly

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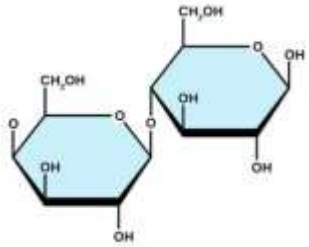
sour taste, pleasant aroma, and smooth consistency, making it a valuable component of a healthy diet.

USES

- ***Improves Digestion*** – Curd contains probiotic bacteria that help maintain healthy intestinal microflora and improve digestion.
- ***Enhances Gut Health*** – The beneficial microorganisms present in curd support gastrointestinal health and reduce digestive discomfort.
- ***Boosts Immunity*** – Regular consumption of curd may strengthen the immune system by promoting a healthy gut microbiome.
- ***Rich Source of Nutrients*** – Curd provides proteins, calcium, phosphorus, and vitamins essential for growth and maintenance of the body.
- ***Prevents Diarrhea*** – Probiotic bacteria in curd help restore the natural balance of gut microorganisms, especially after antibiotic use.
- ***Supports Bone Health*** – Its high calcium content helps in the development and maintenance of strong bones and teeth.
- ***Used in Probiotic Formulations*** – Curd serves as a natural source of probiotic microorganisms and is often used in probiotic research and product development.
- ***Food Ingredient*** – It is widely used in preparing various foods such as yogurt-based drinks, desserts, curries, and fermented products.

EXCIPIENTS

1. LACTOSE

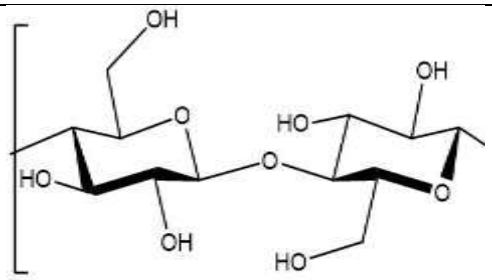


Molecular Formula:- *$C_{12}H_{22}O_{11}$*
Molecular Weight:- *342.30 g/mol

USES:-

* Used as an energy source in milk and dairy products.
* Widely used as a diluent and filler in pharmaceutical tablets and capsules.
* Serves as a carrier for dry powder inhalation formulations.
* Used in infant formulas and nutritional supplements.
* Acts as a substrate for fermentation by probiotic microorganisms.

2. MICROCRYSTALLINE CELLULOSE



Molecular Formula:- *$(C_6H_{10}O_5)_n$*.

Properties

* Appearance: White crystalline powder
* **Odor:** Odorless
* **Taste**: Tasteless
* **Solubility**: Insoluble in water; swells in water
* **Source:** Purified cellulose from plant materials

Advantages:-

* Excellent compressibility
* Chemically stable and inert
* Non-toxic and biocompatible
* Suitable for direct compression processes

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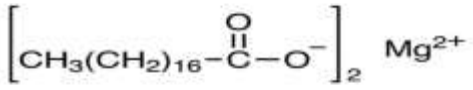
3. MAGNESIUM STEARATE



Properties

* **Appearance**: Fine white powder
* **Odor**: Odorless
* **Taste:** Slightly characteristic
* **Solubility:** Practically insoluble in water and alcohol
* **Stability**: Stable under normal storage conditions

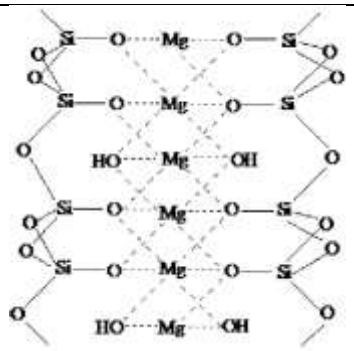
Advantages:-

* Excellent lubrication efficiency
* Improves manufacturing process and product quality
* Effective at low concentrations (usually 0.25–5%)
* Non-toxic and widely accepted in pharmaceutical formulations

Molecular Formula: - Mg(C_{18}H_{35}O_{2})_{2}

Molecular Weight:- *591.24 g/mol*

4. TALC



Properties:-

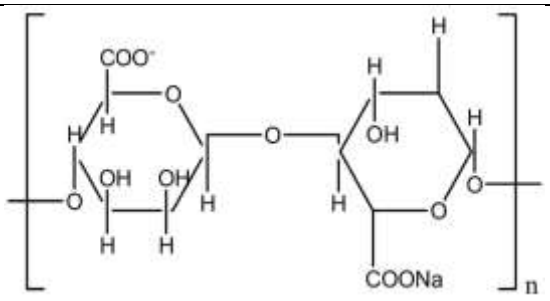
* **Appearance**: Fine white or grayish-white powder
* **Odor**: Odorless
* **Solubility**: Insoluble in water and most organic solvents
* **Nature**: Soft, smooth, and chemically inert

Advantages

* Enhances powder flow ability.
* Prevents sticking during tablet compression and capsule filling.
* Chemically stable and non-reactive.
* Effective at low concentrations.

Molecular Formula: - Mg_{3}Si_{4}O_{10}(OH)_{2}

5. SODIUM ALGINATE



Properties

* **Appearance**: White to yellowish-white powder
* **Odor**: Odorless
* **Solubility**: Freely soluble in water; insoluble in alcohol
* **Nature**: Hydrophilic and gel-forming
* **Source**: Extracted from brown algae (seaweed)

Advantages

* Biocompatible and biodegradable

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	* Non-toxic and safe for pharmaceutical use * Excellent gel-forming ability in the presence of calcium ions * Protects sensitive ingredients such as probiotics from environmental stress **Molecular Formula**: - (C_{6}H_{7}NaO_{6})_{n}

2. METHODOLOGY

- **PREPARATION OF PROBIOTIC POWDER:**
- **Step 1: Selection of Curd**

  Fresh, unpasteurized curd is selected as it contains a high number of live probiotic bacteria.
- **Step 2: Dilution**

  Take 10 g of curd and mix with 90 ml of sterile distilled water.
  Shake well to obtain a uniform suspension.
- **Step 3: Filtration**

  Filter the mixture using a muslin cloth to remove solid particles.
- **Step 4: Isolation of Probiotic Bacteria**

  Spread the filtrate on MRS agar plates.
  Incubate at 37°C for 24–48 hours.
  Colonies of Lactobacillus will appear.
- **Step 5: Collection of Biomass**

  Collect the bacterial colonies using a sterile loop.
  Transfer into sterile saline solution.
- **Step 6: Concentration**

  Centrifuge the suspension at 3000–5000 rpm for 10–15 minutes.
  Discard the supernatant and collect the pellet (bacterial mass).
- **Step 7: Drying**
- **Two methods**

  Freeze Drying (Best Method)
  Freeze the bacterial suspension and dry under vacuum.
  Maintains maximum viability.
- **Oven Drying**

  Dry at low temperature (≤40°C) to avoid killing bacteria.
- **Step 8: Powder Formation**

  Grind the dried mass using a mortar and pestle.
  Store in an airtight container with desiccant.

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Fig. 5 .1- Selection of curd.

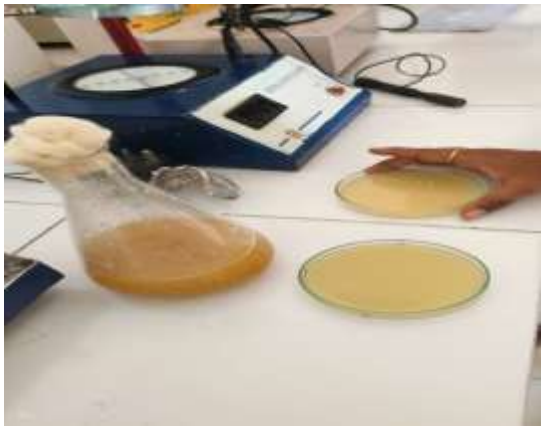

Fig. 5 .2- Preparation of agar plates.

Fig. 5 .3- Inoculation on agar plates.

Fig:5.4-incubation.

Fig:5.5-Centrifuge

Fig:5.6-Drying.

Fig:5.7-Powder

FORMULATION & DEVELOPMENT

1. Dry blending of Probiotic powder with excipients under asept.
2. Encapsulation using hard gelatin capsules.
3. Optimization of formulation for uniform weight and viability.

Fig. 5.8- INGREDIENTS

Fig :- 5.9- Dry granulation of excipients with probiotic powder

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Fig 5.10: encapsulation of gelatin capsules

FORMULATION TABLE

INGREDIENTS	F1 (mg/capsule)	F2 (mg/capsule)
Lactobacillus acidophilus	50 mg(≈ 1×10^9 CFU)	50mg (0.05g)
lactose	120 mg (0.12)g	100mg (0.1gm)
Microcrystaline cellulose	50 mg	70 mg(0.07)g
Magnesium stearate	2 mg (0.002)g	2mg
Talc	3 mg (0.003 g)	3mg
Sodium alginate	-	20 mg (0.02 g)
Total	225 mg	245mg

ORGANOLEPTIC PROPERTIES

- **Appearance:-**Fine, free-flowing powder
- **Color: Off-white** to cream or light yellow
- **Odor:-**Mild characteristic fermented odor; no foul smell
- **Taste: Slightly** acidic or bland (if evaluated)
- **Texture: Smooth**, dry, non-gritty, and free from lumps

RESULTS AND DISCUSSION

1. WEIGHT VARIATION TEST

CAPSULE NO	WEIGHT (MG)	DEVIATION %	RESULT
1	243	-0.82%	pass
2	247	+0.82%	pass
3	244	-0.41%	pass
4	248	+1.22%	pass
5	245	0.02%	pass

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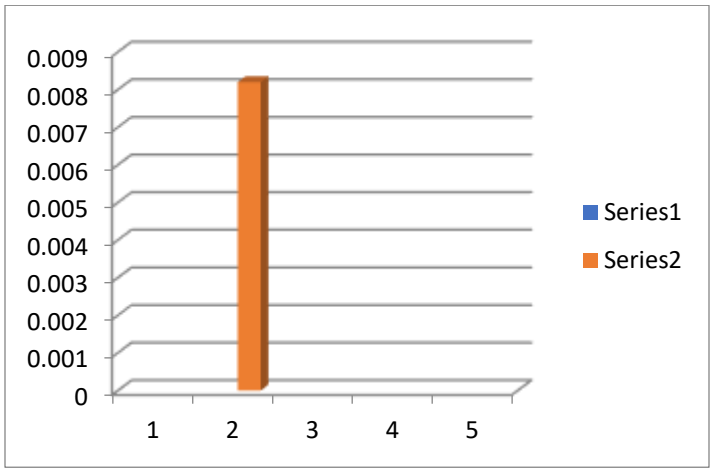

Fig 6.1 Weighing of capsules

2. DISINTEGRATION TEST

CAPSULE	DISINTEGRATION TIME	RESULT
1	6.2	pass
2	6.5	pass
3	6.0	pass
4	6.3	pass

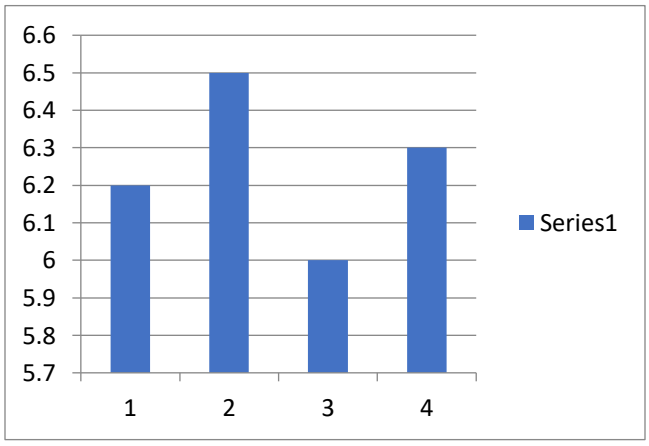

FIG 6.2 DISINTEGRATION

2. MOISTURE CONTENT TEST

SAMPLE	INITIAL WEIGHT (G)	FINAL WEIGHT(G)	MOISTURE CONTENT (%)
1	2.000	1.944	2.80
2	2.000	1.946	2.70
3	2.000	1.942	2.90

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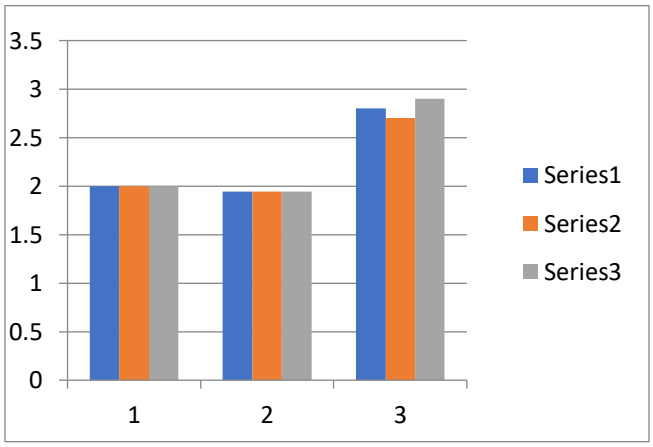

FIG NO: 6.3 MOISTURE CONTENT DETERMINATION.

3. COLONY FARMING UNIT (CFU) COUNT:

TEST	SPECIFICATION	RESULT	STATUS
Total viable count (cfu)	$\geq 1 \times 10^{10}$ CFU/capsule	$\geq 9 \times 10^{9}$ CFU/capsule	Pass
After the stability study	1.05×10^{10} CFU/capsule	9.4×10^{9} CFU/capsule	Pass

DISCUSSION

The present study focused on the development and stability evaluation of probiotic capsules containing beneficial microorganisms. The formulation was prepared using suitable excipients that ensured good flow properties, uniform filling, and adequate protection of the probiotic cells during processing and storage. Preformulation studies showed that the probiotic powder possessed acceptable organoleptic characteristics, moisture content, and flow properties, making it suitable for capsule formulation. The excipients used were compatible with the probiotic strain and did not produce any visible physical changes during the study period. The prepared capsules complied with pharmacopeial quality standards. Weight variation, disintegration time, and moisture content remained within acceptable limits, indicating uniformity and good manufacturing quality. These results suggest that the selected formulation and encapsulation process were appropriate for producing stable probiotic capsules.

Stability studies conducted under recommended storage conditions demonstrated that the capsules maintained their physical appearance, capsule integrity, and acceptable viability of probiotic microorganisms throughout the study period. A slight reduction in viable cell count was observed during storage, which is expected because probiotics are living microorganisms. However, the reduction remained within acceptable limits, indicating that the formulation provided adequate protection against environmental stress. The results are consistent with previous studies reporting that low moisture content, suitable capsule shells, and proper storage conditions improve the stability and shelf life of probiotic formulations. Maintaining viability throughout storage is essential to ensure that an adequate number of live microorganisms reach the gastrointestinal tract and provide the intended health benefits. Overall, the developed probiotic capsule formulation demonstrated satisfactory physicochemical properties and acceptable stability. The findings suggest that the formulation is suitable for oral administration and has the potential for commercial development. Further long-term stability studies and in vivo evaluation are recommended to confirm shelf life and therapeutic efficacy.

SUMMARY

The present study focused on the development and stability evaluation of probiotic capsules containing viable beneficial microorganisms. Preformulation studies were carried out to assess the physicochemical properties of the probiotic powder, including organoleptic characteristics, moisture content, flow properties, and compatibility with selected excipients. Based on these findings, an optimized capsule formulation was developed using suitable excipients to ensure uniformity, stability, and ease of manufacturing.

The prepared capsules were evaluated for various quality control parameters, including appearance, weight variation, moisture content, disintegration time, microbial viability, and content uniformity. The formulation met the acceptable pharmaceutical standards and demonstrated satisfactory physical and microbiological characteristics.

Stability studies were conducted under recommended storage conditions to evaluate the effect of time and environmental factors on the quality of the probiotic capsules. During the study period, the capsules showed no significant changes in physical appearance, moisture content, or capsule integrity. The viable probiotic count remained within acceptable limits, indicating that the formulation effectively maintained the stability and viability of the probiotic microorganisms.

Overall, the study demonstrated that the developed probiotic capsule formulation is stable, safe, and suitable for oral

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administration. The findings suggest that proper formulation design, appropriate excipients, and suitable packaging play a crucial role in preserving probiotic viability and ensuring product quality throughout its shelf life. This work provides a useful foundation for the future development and commercialization of stable probiotic capsule formulations.

CONCLUSION

The present study successfully developed probiotic capsules with satisfactory pharmaceutical and stability characteristics. Appropriate formulation and manufacturing techniques were used to prepare capsules with acceptable organoleptic properties, good flow characteristics of the powder blend, uniform weight, suitable moisture content, and proper disintegration time. The formulated capsules met the required quality control parameters, indicating their suitability for oral administration. Stability studies conducted under the recommended storage conditions demonstrated that the probiotic capsules retained their physical appearance, viability, and overall quality throughout the study period. No significant changes were observed in the evaluated parameters, confirming that the formulation remained stable and effective during storage. The use of suitable excipients and protective packaging contributed to maintaining probiotic viability and product quality.

Overall, the findings indicate that the developed probiotic capsule formulation is stable, safe, and pharmaceutically acceptable. The study underscores the importance of optimized formulation design and appropriate storage conditions for preserving probiotic viability and therapeutic potential. Further long-term stability studies and clinical evaluations are recommended to confirm the product's efficacy, shelf life, and potential for large-scale commercial application.

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