Research Article

Challenges in Protein Formulation Focused on Extrusion-Spheronization Process

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ABSTRACT

Biotechnology revolution had led the overcoming of different types of therapeutic protein because of their chemical structure can perform specific reactions in the body, increasing efficacy and decreasing side effects. Numerous efforts were made to optimize the physicochemical properties of the proteins used for therapeutic and studied different methods for an effective administration of the protein contained in the medicine, evaluating different routes of administration to achieve the desired therapeutic effects. The delivery system for oral pharmaceutical proteins and peptides is still in development stage. There are number of limitations to oral delivery of proteins such as barriers to peptide bioavailability after oral administration, intestinal membrane permeability, size, intestinal and hepatic metabolism and solubility. Pellets have shown great potential in the delivery of proteins/peptidal drugs. Some strategies of development of oral protein and peptides has always been challenged, optimizing the safety and efficacy while ensuring the ability to manufacture the drug while maintaining quality and stability. The pelletization techniques have been reviewed in numerous papers, is a technique that enables the formation of spherical beads or pellets with a mean diameter usually ranging from 0.5-2.0 mm. Pellets are prepared by different techniques, such as extrusion and spheronization. This review discusses challenges in protein formulation that have been used to prepare pelletized dosage forms using the extrusion-spheronization process.

Keywords: Drug delivery, multiple-unit system, extrusion-spheronization, pellets, formulation

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I. INTRODUCTION

In the drug discovery, drug delivery plays a fundamental role in human and animal health care industry [1,2]. The term drug delivery is a concept integrated between dosage form, molecular design or other physical- chemical approaches sustained on the basis of metabolism, pharmacokinetic and pharmacodynamic relationships [2,3]. The objectives of any delivery system is to improve or facilitate the action of the compounds with therapeutic unsafely work. A drug delivery system is ideal when delivering the correct amount of drug to the site of action at the speed and at the right time, maximizing the desired therapeutic response. The evolution of a drug from the traditional way of releasing a new delivery system can significantly improve their performance in terms of efficacy, safety and compliance with therapy by the patient [4,5]. Routes of administration depend on clinical needs and circumstances, so drugs can be introduced into the organism in a variety of tract. Traditionally, called routes of administration have been divided into two major classes, enteral (based on the intestine) and parenteral (means different from the intestine). The route of administration chosen can have a marked effect on the speed and efficiency with which acts the drug. In addition, adverse effects due to the drug itself and the means of

administration are influenced by the way. In this context, oral delivery is the most convenient and comfortable route of administration for most of the drugs and pharmaceutical products. Bioavailability is dependent upon the molecular mass of drugs. The bioavailability of drugs markedly decreases when the molecular weight increases above 500-700 Da, while bioavailability is essentially independent of the molecular-mass products less than 600 Da [6-8].

Many drugs and most widely used formulations for new and existing modified release products prefer the oral solid dosage as route of administration.

In the drug discovery process, a large proportion of novel chemical entities and many existing drug molecules exhibit poor water solubility and hence poor oral absorption, as proteins for using in therapeutic [9,10]. The design and composition of dosage forms marketed controlled release present differences and can be classified as either single unit or multiple unit dosage forms. The single unit dosage forms (non-divided formulation) usually refer to diffusion controlled system where the drug is dissolved or dispersed throughout a solid matrix and the release of the drug is controlled or sustained either by incorporating a suitable filler within the matter or by coating the matrix with swellable or non-swellable polymer film(s) [6-8]. Among the many controlled release formulations for use existing oral drugs, multiple unit systems are being widely used and have generated great impact on the market. Chemicals of low molecular weight are being replaced by the use of different therapeutic proteins because of their specificity and potency.

The revolution of biotechnology has led to the emergence of different types of therapeutic proteins, providing alternative therapeutics to certain diseases that were considered incurable. Currently biotherapy has the potential to target chronic and malignant diseases such as cancer, autoimmune disorders, etc. Furthermore, proteins can be used to detect and diagnose disease, and as vaccines in order to ensure the protection of various diseases. Others, bacterial antagonism that synthesized peptides, generally referred to bacteriocins, has gained considerable attention because of its potential applications in the control of undesirable microbiota. The chemical structure of proteins allows them to perform specific reactions in the body, increasing efficacy and decreasing undesirable side effects. Among the obstacles that are faced by proteins that are used for therapeutic purposes is their short life blood, so it is necessary to repeat administrations, their chemical composition and physical instability, rapid denaturation in the stomach and the intestinal environment, and their retention in the impermeable mucosal tissues gut, making it difficult to administer proteins orally.

The above limitations impose restrictions for the development of proteins as pharmaceuticals. That is why the procedures for obtaining them should be controlled and the administration system should be adequate, minimizing aggregation and viscosity of the proteins [9,10] Some specific factors as high molecular weight, *in vivo* and *in vitro* instability, immunogenicity and shorter half-live limit the safety and efficacy of protein and peptide therapeutics [8-11]. Various techniques have been developed to improve the physicochemical properties of proteins, protect them in biological media and deliver them to their target [12-23]. This review describes some of the techniques that have been used to achieve the objectives described above, such as the structural modification of the protein, the use of special assistants that can improve the absorption of protein and inhibit protein degradation. The possible therapeutic applications of proteins and peptides and the obstacles that limit their widespread clinical use in human and veterinary medicine is also highlighted.

The main objective of this work was to investigate the application of the method of extrusion spheronization as a drug delivery system for therapeutic proteins. Oral administration of proteins and their physicochemical properties have been insufficient scientific major challenges in the area of the formulation, thus resulting in the need to explore other routes of administration such as nasal, pulmonary, transdermal, rectal and ocular. Without an effective route and method of deliver, the use of proteins and peptides as therapeutic agents is limited. Within the areas of development related to therapeutic proteins

and peptides that have been relevant for scientists it includes the following items:

- In almost every field of human and veterinary medicine, the high molecular weight of the therapeutic molecules play an important role, for example enzymes, structural elements, hormones or immunoglobulins.
- Proteins and peptides are complicated hydrophilic macromolecules and these characteristics complicate to enter into cells and other body compartments.
- Many therapeutic proteins and peptides show efficiency in part because of their tertiary structure, which can be lost under different physical and chemical environments, losing the biological activity, hence, making these biomolecule inherently unstable. The biological half-time in the blood is short due to clearance from the bloodstream, resulting necessary repeat administration in order to maintain therapeutics levels in the blood.
- A precise clinical dosing is important for proteins and peptides because of their potency and specificity.
- Some post-translational modifications (glycosylation, phosphorylation and proteolytic cleavage) are necessary for a protein which is physiologically active.
- Because the technologies involved in the design and development of therapeutic proteins and peptides, it turns a costly final product.
- An important obstacle to overcome in the absorption of a therapeutic protein is its penetration through the mucosa to reach the bloodstream. A hydrophilic high molecular weight molecule is degraded quickly by mouth, so it will not be available in the bloodstream.

For example, Aoki et al demonstrated through his *in vitro* studies That the mucus layer plays a critical role in the absorption of insulin across the small intestinal.

2. MULTIPLE UNIT SYSTEM

In the early 1950s the concept of multi-unit dosage was introduced. Multiparticulates are defined as discrete drug particles combined into one dosage unit to form a multiple-unit system having a diameter ranging from about 0.3-1.5 mm [22-24]. Multiparticulate dosage forms are pharmaceutical formulations in which the active ingredient is present as a number of small independent subunits. To administer the recommended total dose, these subunits are introduced into a sachet and encapsulated or compressed into a table. These dosage forms usually are based on subunits such granules, crystal, minitablets, sugar seeds, ion- exchange resin particles, powders or pellets.

Advantages and disadvantages of multiple-unit systems

Multiple-unit systems have several advantages over single-unit systems: greater flexibility of dosage form design and development, ease of coating, ease of capsule filling, improved elegance, product identification and patient compliance, controlled delivery of drugs for oral use, ease of design of controlled-release formulations containing more than one drug, ease of drug dissolution and analysis, greater stability of chemically incompatible drugs, ease of dose divisibility, greater safety and efficacy of drugs (pellets are mainly coated in order to either sustain drug release or to deliver a drug to the specific absorption site in the gastrointestinal), lowered tendency for dose dumping, reproducibility of plasma profile and therapeutic effect and marketing edge.

Some of the potential limitations of multiple-unit formulations are:

- Production of pellets is often an expensive process and/or requires highly specialized equipment compared to simple agglomeration-granulation.
- The control of the production process is difficult, quite often the pellets cannot be compressed into tablets because they are too rigid.
- The coated multiparticulates, when compressed into tablets (called MUPS) often lose the purpose of applied coating owing to damage to coating as a result of compression forces [22-24].

The modified-release coated multiparticulates formulations could be design by granulation, spray drying, spray congealing and pelletization.

Pellets as a solid dosage form

Pharmaceutical industries applicant for the production of pellets some different techniques, which are:

a) Solution and suspension layering

This process uses conventional coating pan or fluidized bed with conventional top spray or Wurster bottom spray to

apply drug/binder solution or suspension to solid cores that can be inert materials or granules of the same drug [17,18].

b) Dry powder layering

This process involves the use of rotor-granulator/tangential rotor fluid bed spray depositing successive layers of dry powder drug and excipients appropriate on inert materials, generally a dissolution liquid adhesive/bonding is used [19,20].

c) Direct powder pelletization

This technique requires the use of high shear mixers and centrifugal fluid bed granulators or rotary fluid bed filled with fluid to apply direct agglomeration a powder mixture of a drug and excipients, followed by granulation through a rotating disk. As an alternative step can add a liquid (wet granulation) a molten binder before or during (melt pelletisation) [21,22].

d) Balling

This technique requires a single step, in which the dusts is converted to spherical granules of various sizes by a continuous rolling or tumbling motion [23,24].

e) Globulation or droplet formation

To generate spherical particles or pellets is necessary to use two processes, agile and freeze dried by spraying, atomizing involving hot melts, solutions or suspensions [25]. The drug is dispersed or dissolved in the polymer solution, polymer dispersion or molten wax followed by spray drying or spray congealing [26,27].

Spray drying is the process in which drugs in the suspension or solution without excipient are sprayed into a hot stream to produce dry and more spherical particles [28,29]. Spray congealing is a process in which a drug is allowed to melt, disperse or dissolve in hot melts of polymers, lipids, waxes or fats. This technique requires higher ratio of coating agents to active material.

f) Compression

Pellets of definitive sizes and shapes are prepared by compacting mixtures or blends of active ingredients and excipients under pressure. The formulation and process variables controlling the quality of pellets prepared are similar to those used in tablet manufacturing [30,31].

g) Cryopelletization

This process is used to produce drug loaded pellets for immediate release formulations.

The initial technique used for lyophilization of viscous bacterial suspensions can be used also for producing drug-loaded pellets to allow the droplets of liquid form such as solution, suspension or emulsion to contact with liquid nitrogen at -1600°C [32,33].

h) Extrusion-Spheronization process

Is a multistage process for obtaining pellets with uniform size from wet granulates [34-38]. Pelletization methods used in the pharmaceutical industry can be grouped by various criteria, for example by the type of equipment used, the intensity of the mechanical forces involved or the techniques employed for the production of pellets. The success of these methods depends on the complex relations between the equipment, the formulation and process variables.

3. EXTRUSION-SPHERONIZATION PROCESS

A large percentage of pharmaceuticals that are administered orally (tablets or capsules) are formulated to release the active substance is immediate. In conventional formulations no effort is made to modify the release rate. However, the products known by the name of immediate release permit rapid absorption of the active ingredient and the onset of pharmacodynamics phase accompanying the expected effects. The pattern of drug release from modified-release dosage forms is deliberately changed from that of a conventional dosage formulation to achieve a desired therapeutic objective or better patient compliance. Pellets are still gaining interest due to their therapeutic and technological advantages such as disperse freely in gastrointestinal tract, maximize drug absorption, minimize local irritation of the mucosa, improve flow properties, less friable dosage form, narrow particle size distribution, ease of coating and uniform packing [34-40]. Extrusion-spheronization is one of the most important methods in pellet production, which involves five unit operations: dry mixing, wet massing, extrusion, spheronization, drying

and screening, strongly related to each other (**Figure 1**).

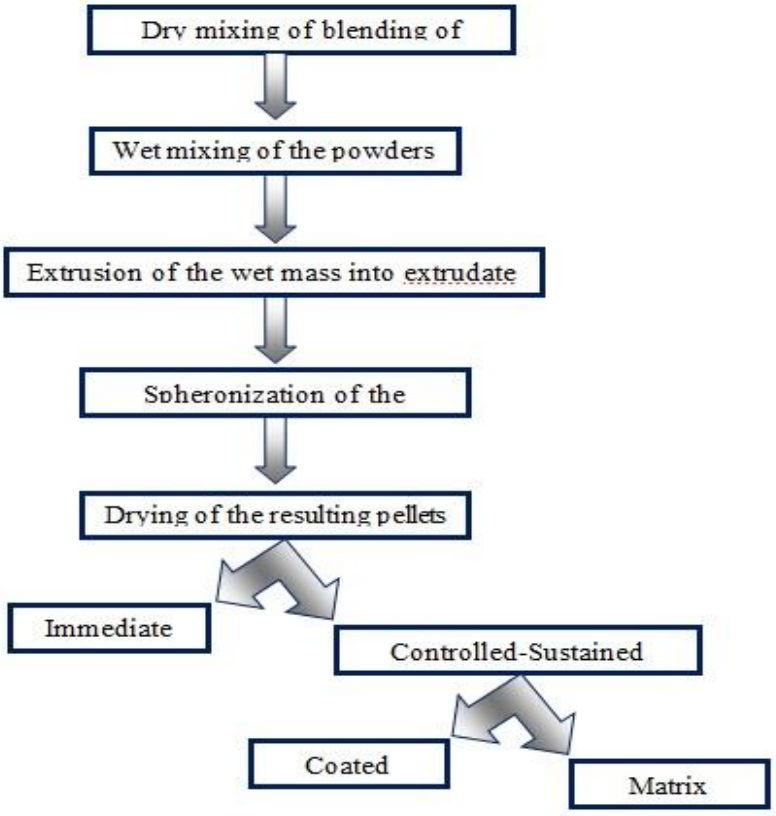

Figure 1: Extrusion-Spheronization process usually involves the following steps.

To achieve a homogenous powder dispersion, it does a dry mixture of all the ingredients using a blender shell mixer, planetary mixer, high speed mixer and twin drum. This wet mixture is conducted to produce enough for extrusion, wet granulation employed in the plastic mass for compaction.

There are two critical variables, the amount of liquid (solvent or binder solution) that is added to the powder mixture to obtain a wet mass, and uniform distribution of the liquid throughout the powder mass that must be appropriately controlled during the mixing process in order to obtain the desired pellets.

Extrusion is the third step in this process where the wet mass is forced to pass through a mold or die of an appropriate opening to create cylinders or rod-shaped masses known as extrudates.

In order to achieve the desired moisture content is necessary to carry out a drying step. To achieve the desired distribution of particle size is essential screening stage. Among the advantages of pellets can be mentioned:

- Pellets disperse freely in gastrointestinal tract, maximize drug absorption, and minimize local irritation of the mucosa by certain irritant drugs.
- After swallowing, adequate dispersion of the particles it is achieved, obtaining a relatively low local concentration. Moreover irritation of the gastrointestinal mucosa is markedly lower.
- Different coatings can be mixed and filled into capsules to achieve the desired dissolution profile.
- When should prepare pellets with incompatible active agents, these coatings permit brought into capsules or compressed into tablets without risk of interaction of substances during preparation and storage. This allows you to administer the drugs as a dosage form a single unit.
- They can be formulated as sustained, controlled, or site-specific delivery of the drug from coated pellets.
- The reduced friability, uniform distribution of particle size and ease of

coating and filling capsules are some of the advantages of manufacturing.
- This technology is used to retain a variety of proteins, probiotics, vitamins, antioxidants, among others.

Parameters affecting quality of pellets in extrusion-spheronization process

There are many factors that could affect the quality of the pellets such as composition and amount of binder, physical properties of starting material, screen hole diameter, extruder speed, mixing time, mixing speed, spheronizer load, time and speed, thickness of the die, drying techniques, drying time and extrusion screen. In (**Table 1**) are summarized the factors that affect the quality of pellets in the extrusion-spheronization process.

Table 1: Parameters that may have impact on the preparation of pellets.

VARIABLES THAT INFLUENCED IN THE QUALITY OF PELLETS	
ENVIROMENT	- Weather - Humidity - Temperature
OPERATOR	- Analytical Error - Calibration Error - Skill
PROCESS	- Drying Method, Time and Temperature - Mixing Time and Method - Spheronization Load, Time and Speed - Extrusion Time and Speed
FORMULATION	- Surfactant and Cosolvent Concentration - Wetting agent - Drug Loading - Binder Type and Concentration - Disintegrant Type and Concentration

Modified-release multiparticulates can be designed in one of the two ways: matrix drug pellets and reservoir or membrane coated drug pellets. Following factors need consideration for the preparation of coated multiparticulates:

1. The characterization of drug scores includes determination of physical properties of the core, physicochemical properties of the drug and core excipients.
2. The characterization of the coat includes determination of physical properties of the coat and the components of the coating.

FORMULATION AND MANUFACTURABILITY OF BIOLOGICS MEDICINES

Many diseases that were considered incurable until recently are used to treat with peptides, proteins and antibodies which have now become important biopharmaceuticals. They can be administered via various routes classified into two major classes: parenteral protein delivery routes (intravenous injection, subcutaneous injection and intramuscular injection) and non-invasive protein delivery routes (nasal, oral, ocular, pulmonary and rectal routes).

When these molecules are *in vivo* poses many problems because of the formulation and specific characteristics, such as short half-life in the bloodstream, the possibility of triggering dangerous immune response and protein instability in the biological medium.

Many technologies have been employed to solve these problems by increasing drug bioavailability, decreasing side effects, increasing ease of use by patients, and reducing the cost of fabrication as much as possible.

An important challenge in the pharmaceutical development of a biologic product is overcoming the difficulties in maintaining protein solutions safe and efficacious throughout the drug product development process.

This manufacturing process consists of several operational steps where the biologic is subjected to different stresses and conditions that may compromise quality and stability [35,40-49].

Strategies used to improve the bioavailability

Penetration enhancers

To facilitate the passage of an adequate amount of pharmacologically active protein, penetration enhancers are used, which can be recognized as peptides or substances through the mucosal membranes [50,51].

Enzymatic inhibitors

The enzymatic barrier is considered to be one of the dominant factors controlling the bioavailability of given proteins and peptides, independently of the route used to administer protein drugs. This is due to the high sensitivity of proteins and peptides to the different types of enzymes, and to the ubiquitous nature of these enzymes in the body [52].

Protein encapsulation as a delivery system

A promising method for delivering these therapeutic molecules is the protection of proteins and peptides into polymeric deposits [8].

Among the advantages provided by these drug delivery systems of the particles, are include the encapsulation protein protection against the effect of enzymes, and the site control the release rate of proteins helping to avoid undesirable side effects [13]. For the encapsulation of therapeutic proteins have been studied and used different techniques.

Protein formulation extrusion spheronization

There are well-described methods for the development of multiparticulate dosage forms for therapeutic proteins and peptides, such as extrusion-spheronization among others.

Because of their low oral bioavailability and propensity of this type of biomolecules, all techniques must be evaluated in terms of enzymatic activity. In the recent years, there have been developed novel synthetic strategies to improve productivity and reduce metabolism of proteins and peptides, along with alternative routes of administration.

Some molecules like hormones, growth factors and peptide inhibitors were mimetized naturally for synthetic peptides analogues. This possibility emerged because their ease of synthesizing large amount of peptides and liberty to modify peptides in order to make them more effective.

Most therapeutic peptides are derived from natural or bioactive peptides produced by plants, bacteria, animal or human, peptides isolated from genetic or recombinant peptide libraries and discovered from chemical libraries. Routes for peptides and proteins with different size are from the chemical or enzymatic synthesis, recombinant DNA technology, systems of cell-free expression and transgenic animals.

Chemical synthesis offers access to a much wider chemical diversity using unnatural amino acids and pseudo-peptide bonds, than peptide derivatives produced by recombinant technologies, with a diversified potential for intellectual property (in terms of patentable new chemical entities).

Absorption, distribution, metabolism and excretion processes play a fundamental role in defining the disposition of a drug candidate and thus its therapeutic effect. Within the parameters crucial for the development of a peptide as a therapeutic agent are its pharmacokinetic profile, low immunogenicity and biological effect.

The chemical optimization strategy of a therapeutic peptide or protein is based on structure activity relationship and/or quantitative structure activity relationship studies of newly synthesized peptide derivatives, with the aim of improving bioavailability, reducing elimination and biodegradation and increasing selectivity or affinity to its receptor or target.

From a larger parent molecule, they found peptide fragments and polypeptides active. The peptides have a simple structure, making them models to study the domains of the largest proteins and which are suitable for drug design. Among the factors contributing to a marked increase in the pharmaceutical application of peptides is the small size, allowing tissue penetration cannot be achieved by larger proteins. Therapeutic peptides currently being applied in different fields of medicine such as diagnosis, oncology, cardiovascular and infectious bacterial disease, diabetes, obesity, arthritis and central nervous system disorders.

The wider and successful use of proteins and peptides as drugs are faced by many obstacles and challenges such as protein *in vivo* instability, poor absorption, and short half-life.

Many strategies have been developed to overcome these challenges and to improve protein drug efficacy, such as permeability enhancement, enzyme inhibition, and protein structure modification and protection.

Therefore, research is focusing on alternatives routes of delivery inhaled buccal, intranasal and transdermal route, as well as novel delivery systems such as the use of protective liposomes. The majority of veterinary compounds being targeted for modified sustained release include antibiotics, antiparasitic agents and hormones. Such compounds have been formulated into technologies that include subcutaneous implantable systems, microspheres and microcapsules, oil-based injectable controlled release formulations, oil-based spot-on formulations that are added directly to the skin, ear tags and collars, ophthalmic and oral topical devices. Ferraz et al. [53]. evaluated the influence of the operational stages, operational conditions, formulation parameters and storage of the final product, involved in the extrusion-spheronisation technique, over papain bioactivity. The results were that extrusion-spheronisation may be applied to produce multiparticulate delivery sytem for oral delivery of therapeutic proteins of interest with high retained activity. Other studies undertaken by Remon et al. [47]. with different formulation techniques, in order to develop multiparticulates formulation of viable interleukin-10 producing Lactococcus lactis Thy 12, concluding that the layering of Thy 12 on inert microcrystalline cellulose pellets was a promising technique being economical and with single steps production process. However, Ghaid et al. [35]. described the extrusion-spheronization technique as one of the most promising process for the optimum delivery of many potent drugs having high systemic toxicity, offering an immense pharmaceutical applicability due to the benefits of high loading capacity of active ingredient(s), narrow size distribution and cost effectiveness [14].

Several single therapeutic peptides have a market size of over a billion dollar, being the veterinary medicine market another target to explore.

Pellets, as a drug delivery system, can provide many advantages and extrusion-spheronization is the most commonly manufacturing technique in the pharmaceutical industry to prepare them.

Traditionally the therapeutic proteins are administered in parenteral administration because stability problems, unlike small chemical molecules which are usually administered orally. Pharmaceutical products containing protein should be stored in cold conditions or lyophilized to achieve acceptable shelf life.

CONCLUSION

The use of protein-based therapeutics such as vaccines, antigens and hormones has increased but biological limitations impose restrictions for the manufacturing and development of protein pharmaceuticals.

Various techniques of drug delivery have been explored, which come in different sizes, shapes, and compositions. The rapidly growing pharmaceutical industry is continuously looking to the development of new active molecules, which inevitably requires a suitable dosage form capable of delivering those molecules effectively in the body.

Pellets as a drug delivery system offer biopharmaceutical advantages and represent a route to overcome limitations of therapeutic proteins. The extrusion-spheronization, the most commonly and effectively process, may be applied to produce multiparticulates delivery systems for oral delivery of therapeutic enzymes and other proteins of interest with high-retained activity. However, biological limitations impose restrictions for the manufacturing and development of protein pharmaceutical, which require controlled procedures and suitable drug delivery systems. The use of therapeutic proteins and enzymes at industrial level has increased and these macromolecules are replacing low molecular weight chemicals due to their high specificity and potency. Pharmaceutical industries will continue to focus on these molecules in association with the development of new oral formulations and delivery technologies.

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