Stony Brook University

Preparation of Microspheres and Nanospheres for Optimum Drug Delivery in Volume Sensitive Applications

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by

Brinda Nikhil Doshi

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In Partial Fulfillment of the

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Abstract of the Thesis

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Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Efforts in the area of drug delivery include the development of targeted delivery in which the drug is released over a period of time in a controlled manner from a formulation. Types of sustained release formulations include loaded biodegradable microspheres and nanospheres.

This research is carried out to achieve successful drug delivery through loaded biodegradable microspheres and nanospheres for volume sensitive applications in the human body. The microspheres can be implanted as compressed tablets in the targeted area of the body; whereas the nanospheres can be injected into the body in a dispersed form in volume sensitive areas of the body. These areas mainly include the retina of the eye where volume is the most important factor. The benefit of using biodegradable polymers is that the polymers degrade inside the body after a period of time and hence there is no surgical removal of any implant necessary, which is patient favorable.

In this study, matrix tablets with poly (lactide-co-glycolide) (PLGA) and poly (lactic acid) (PLA) particles and drug pellets are prepared by direct compression to evaluate the effects of altering PLGA/PLA particle size and compression pressure on the release rates of the drug from matrix tablets. Different release mechanisms based on the end application are also proposed and discussed.

PLGA/PLA microparticles and nanoparticles were prepared by a modified in-emulsion-solvent-evaporation method. Tablets were prepared by direct compression of

the particles recovered. The tablets' physical characteristics including weight, dimensions and density were examined. The tablets were then used for a drug release study. A UV plate reader measured the amount of drug released. The readings were then converted into concentration and the sustained drug release profiles were obtained. The profiles for microspheres and nanospheres are compared along with the different compression pressures used and the different release procedures based on the method of drug delivery to be used.

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INTRODUCTION:

Microencapsulation is one of the most intriguing fields in the area of drug delivery systems. It is an interdisciplinary field that requires knowledge of the field of pure polymer science, familiarity with emulsion technology, and an in-depth understanding of drug and protein stabilization. It is a technology devoted to entrapping solids, liquids, or gases inside one or more polymeric coatings. (10).

Two general structures exist: microcapsules and microspheres. A microcapsule is a system that contains a well-defined core and a well-defined envelope: the core can be solid, liquid or gas; the envelope is made of a continuous, porous or non-porous, polymeric phase. A microsphere is a structure made of a continuous phase of one or more miscible polymers in which particulate drug is dispersed, at either the macroscopic (particulates) or molecular (dissolution) level. The difference between the two systems is the nature of the microsphere matrix, in which no well-defined wall or envelope exists. (10).

Nanoparticles are solid colloidal particles ranging in size from 10 to 1,000 nm. They are made of a macromolecular material, which can be of synthetic or natural origin. Depending on the process used for their preparation, two different types of nanoparticles can be obtained, namely nanospheres and nanocapsules. Nanospheres have a matrix type structure in which a drug is dispersed, whereas nanocapsules exhibit a membrane wall structure with an oily core containing the drug. Because these systems have very high surface areas, drugs may also be adsorbed on their surface. (10).

Numerous methods exist for the manufacture of microspheres and nanospheres, allowing extensive modulation of their structure, composition, and physiochemical properties. The choice of the manufacturing method essentially depends on the raw material and on the solubility characteristics of the active compound captured by the particles. Regarding the raw material, criteria such as biocompatibility, the degradation behavior, choice of the administration route, desired release profile of the drug, and finally, the type of biomedical application determines its selection. From these considerations, it is clear that particle formulation requires an initial and very precise definition of the needs and objectives to be achieved. (10).

Particles can be prepared by two methods: (1) In Situ Polymerization (2) Dispersion of a preformed polymer.

The latter is the preferred method for making particles for a variety of reasons. Most of the carriers produced by polymerization have inadequate biodegradability properties precluding their use for regular therapeutic administration. In addition, due to the multicomponent nature of the polymerization media, it is generally very difficult to predict the molecular weight of the resulting polymerized material. This is a major drawback because the molecular weight greatly influences the biodistribution and release behavior of the polymeric carrier. Another drawback is the possible inhibition of drug activity due to the interactions with the activated monomers or with the numerous H^+ ions present in anionic polymerization processes. Finally, the main limitation to the use of polymerized carriers is related to the presence of toxic residues, namely the unreacted monomer, the initiator, and the surfactant molecules whose elimination requires time-consuming and not always efficient procedures. (10).

In order to circumvent those limitations and extend the manufacturing possibilities for the achievement of biodegradable, well-characterized, and non-toxic particles, new methods involving the use of already polymerized materials have been developed. These materials include natural macromolecules (biopolymers) and synthetic polymers. Synthetic biodegradable polymers are now most commonly used for the preparation of particles. (10).

Among the numerous synthetic polymers available for the preparation of particles, the most commonly used are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA). Belonging to the family of polyesters, these polymers are known to exhibit adequate biodegradability and biocompatibility. Under physiological conditions, polyesters are generally degraded by hydrolysis into products, which are well tolerated by various tissues. For example, the degradation products of PLA, PGA, PLGA, namely glycolic acid and lactic acid, are physiologic substances easily eliminated through the Krebs cycle. The safety of polyesters is illustrated by their extensive use as sutures and orthopedic implants for approximately two decades, and more recently by their use as controlled delivery devices for drugs. Being directly related to the rate of degradation; the drug release capabilities of polyesters can be easily tailored, depending on the monomer composition and the polymerization conditions. Consequently, polyesters make possible the preparation of delivery systems from which the drug is released in a controlled way, over days to months. There are a huge and increasing number of encapsulation processes. The different types of encapsulation methods are: (10).

1. Interfacial Polymerization
2. Complex coacervation
3. Coacervation
4. Thermal denaturation
5. Salting-out
6. Solvent evaporation
7. Hot melt
8. Solvent removal
9. Spray-drying
10. Phase separation

In this study, microspheres and nanospheres are formulated by the in-emulsion-solvent-evaporation method. It is the most popular way to accomplish encapsulation. A core material is briefly dissolved in a water-immiscible, volatile organic solvent and the resulting solution is emulsified in an aqueous solution. The solvent is allowed to evaporate, thereby producing solid microspheres or nanospheres.

The solvent evaporation encapsulation process is a way of precipitating small polymer particles from an oil-in-water emulsion. The polymer is dissolved in a volatile organic solvent that is immiscible with water. Methylene chloride is a preferred solvent because of its high volatility and its capacity for dissolving a broad range of polymers. There are a number of solvents that can be used, although many solvents suitable for this process have a finite degree of water solubility, even though they are normally classified as water- insoluble solvents. Mixed solvents can also be used. The mixtures used so far

tend to contain a water-immiscible solvent and a water-miscible solvent. The water immiscible solvent is the predominant component of the mixture. (10).

Once the desired coating polymer is dissolved in the organic solvent, the drug to be encapsulated is added to this solution. The drug agent may be a solid (crystalline or amorphous) or a nonvolatile liquid. The added drug may completely dissolve in the polymer solution or it may be completely insoluble and simply form a dispersion, suspension or suspension-emulsion. The solubility of the drug in the organic solvent is also a major factor in determining the morphology of microspheres produced by the solvent evaporation process and the final state of the polymer itself (crystalline or amorphous). (10).

The drug/polymer/solvent mixture (i.e. the oil phase) is emulsified in water to form an oil-in-water emulsion. The size of the oil phase droplets obtained is determined by how rapidly the system is agitated when the oil phase is added to the aqueous phase, and determines the size of the particles produced. Emulsification is carried out in a blender if small particles are desired or with a suspended agitator for larger particles. In order to aid emulsification, a surfactant is normally dissolved in the water phase before the oil-in-water emulsion is formed. A good example is partially hydrolyzed (88%) poly(vinyl alcohol) (PVA). (10).

Once the desired oil phase droplet size and emulsion stability have been obtained the system is stirred at a constant rate and the solvent evaporates. This is the basis of the name, because most of the solvent disappears by evaporation. Evaporation can occur in an open system at reduced pressure and range of evaporation temperatures can be used. Once solvent evaporation appears to be complete, the particles are separated from the suspending medium by filtration, washing and drying. If particles are stored as aqueous suspensions, degradation and/or solubilization of the polymer, drug leakage, drug desorption, and/or drug degradation may occur. Freeze-drying (lyophilization) probably represents one of the most useful methodologies to ensure the long-term conservation of polymeric particles. This technique involves the freezing of the suspension and the subsequent elimination of its water content by sublimation under reduced pressure. After complete dessication, particles are obtained in the form of a dry powder that is easy to handle and to store. Freeze-dried particles are usually readily redispersable in water without modification of their physiochemical properties, because particles are usually produced with surfactants or stabilizers; the residual presence of these compounds generally favors the redispersion of the particles. However, in some specific cases, full redispersion of the system may be difficult to achieve. (10).

The maximum drying temperature must remain below the glass transition temperature of the polymer encapsulant or the particles fuse together. Although most of the process depends on evaporation, some of the solvent may partition into the aqueous solution and then evaporate. The amount that partitions into the aqueous solution directly depends on the solubility of the organic solvent in water.

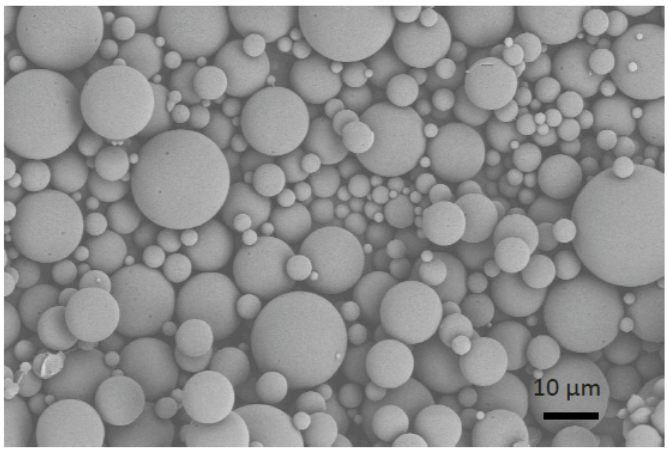
The solvent evaporation process is conceptually simple, but a large number of process variables exist which can profoundly affect the nature of the product obtained. The parameters affecting solvent evaporation are:

1. Polymer molecular weight and concentration
2. Polymer crystallization
3. Type of drug and method of incorporation (solid, liquid, suspension)

4. Organic solvent used
5. Type of surfactant in aqueous phase
6. Organic solvent/aqueous phase cation
7. Evaporation temperature
8. Rate of stirring

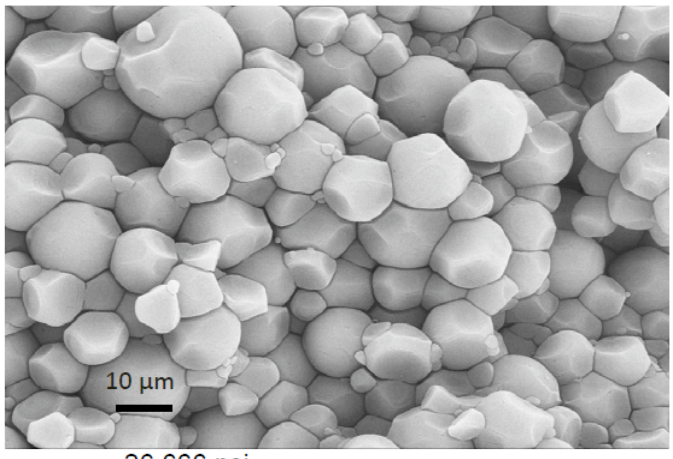
Each of these variables affects the given system differently, which can be determined experimentally; although some general trends are known. (10).

The particles are then compressed in to tablets at different compression pressures and their drug release profiles are observed and studied. Here are some SEM images of particles in their powder form and particles, which are compressed at various pressures.



Native state

Fig. 1: SEM image of a microparticle in its dry powder form



26,666 psi

Fig. 2: SEM image of a microparticle tablet compressed at 26,666 psi

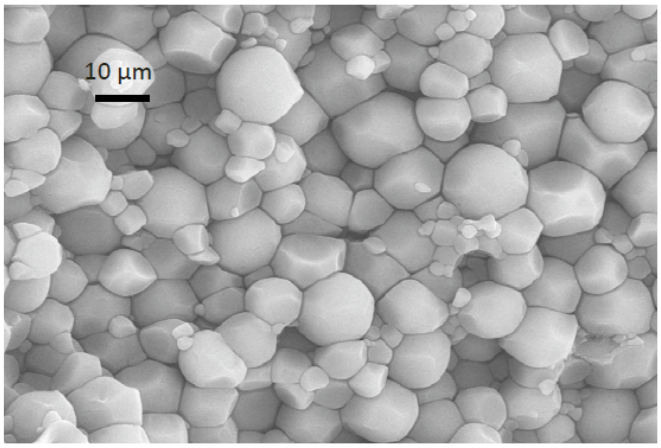


Fig. 3: SEM image of a microparticle tablet compressed at 53,333 psi

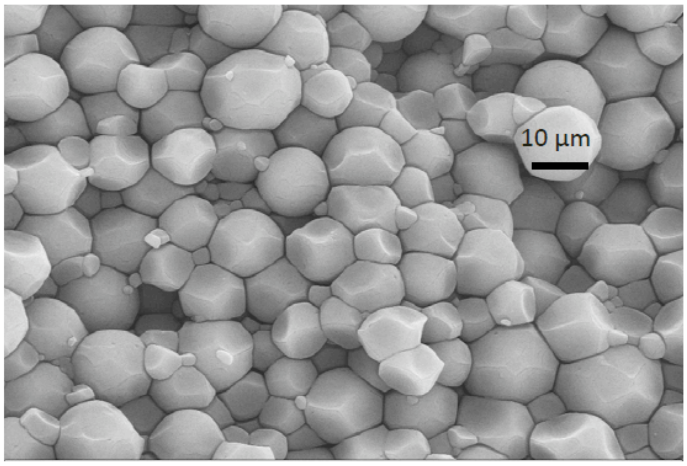


Fig. 4: SEM image of a microparticle compressed at 106,666 psi

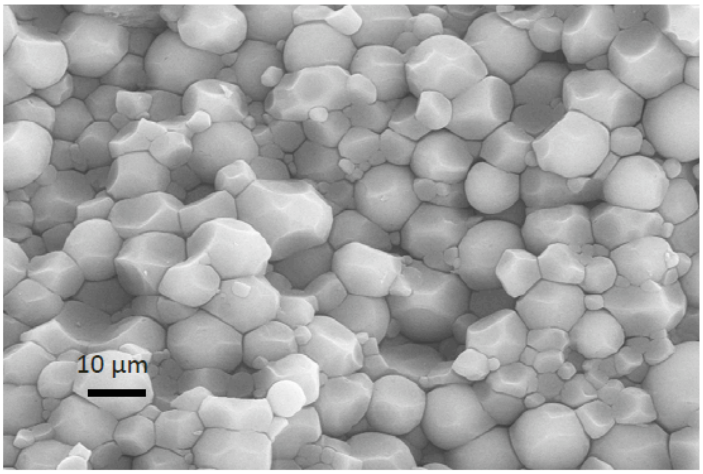


Fig. 5: SEM image of a microparticle tablet compressed at 319,998 psi

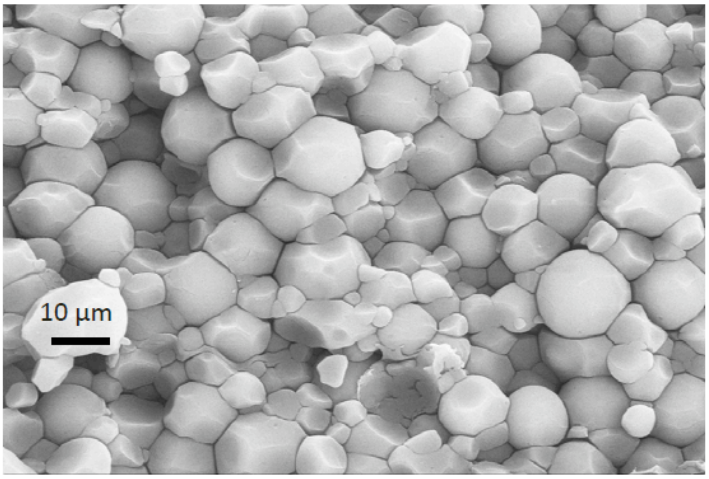


Fig. 6: SEM image of a microparticle tablet compressed at 213,333 psi

MATERIALS AND METHODS:

1) Materials:

PLA (Durect; Lactel) and PLGA (Boehringer Ingelheim; 'Resomer' RG 502H, with 50:50 copolymer ratio of lactide to glycolide), PVA (polyvinyl alcohol) (Sigma Aldrich, P8136-250G; Batch # 086K0021, Average MW 30,000-70,000) and PBS (Roche; 10X PBS, Lot # 1666789) were used as received. Dexamethasone (Spectrum Chemical Mfg. Corp., DE 121, Lot # YT 3030) is used as the active ingredient/drug in this study. The volatile organic solvent used is chloroform (J.T.Baker, 9180-01).

2) Preparation of microspheres:

For this study, 2 batches of microspheres were prepared.

Batch #1:

5 g of PLA is dissolved in 125 ml of chloroform. 325 mg of dexamethasone is added to the solution. The drug/polymer/solvent mixture (i.e. the oil phase) is emulsified in 700 ml of 2% PVA solution. (The solution is prepared by dissolving 14 g of PVA powder in 700 ml of water). PVA is normally added as a surfactant and dissolved in the water phase before the oil in water emulsion is formed, to aid emulsification. A motorized stirrer (Fisher Scientific, Lab-Stirrer LR 400D) at 2200 rpm agitated the system for 90 minutes to obtain the desired oil phase droplet size and emulsion stability. The system is then constantly stirred with an impeller (1/2" diameter) at 2000 rpm till the solvent (chloroform) evaporates. The system is placed in an ice bath during the duration of the agitation. After all the solvent has evaporated, the microspheres are obtained by centrifuging the solution at 2000 rpm for 10 minutes in a centrifuge (Beckman Coulter, Allegra X-15R; Rotor # SX 4750/SX4750A). The microspheres are then lyophilized in a lyophilizer (Virtis Freezemobile 6ES), to obtain them in powder form.

Tableting:

The microspheres of batch # 1 were then compressed into tablets using a compression press (Carver press; Model # 4350.L) and a custom made stainless steel mold with diameter of 8 mm. All the samples weighed about 220-240 mg. The microspheres were compressed at 100,000 psi, 200,000 psi and 300,000 psi.

Particle size measurement:

The mean diameter of the compressed microspheres of batch # 1 in aqueous dispersion for each compression pressure was measured using a light scattering instrument (Beckman Coulter, Model # LS 13320).

Batch # 2:

4 g of PLGA is dissolved in 100 ml of chloroform. 260 mg of dexamethasone is added to the solution. The drug/polymer/solvent mixture (i.e. the oil phase) is emulsified in 500 ml of 2% PVA solution. (The solution is prepared by dissolving 10 g of PVA powder in 500 ml of water). PVA is normally added as a surfactant and

dissolved in the water phase before the oil in water emulsion is formed, to aid emulsification. A motorized stirrer (Fisher Scientific, Lab-Stirrer LR 400D) at 2000 rpm agitated the system for 30 minutes to obtain the desired oil phase droplet size and emulsion stability. The system is then constantly stirred with an impeller (1" diameter) at 1200 rpm till the solvent (chloroform) evaporates. The system is placed in an ice bath during the duration of the agitation. After all the solvent has evaporated, the microspheres are obtained by centrifuging the solution at 2500 rpm for 10 minutes. The microspheres are then lyophilized to obtain them in powder form.

Tableting:

The microspheres of batch # 2 were then compressed into tablets using a compression press (Carver press; Model # 4350.L) and a custom made stainless steel mold with diameter of 8 mm. All the samples weighed about 200-220 mg. The microspheres were compressed at 100,000 psi and 300,000 psi.

Particle size measurement:

The mean diameter of the freeze-dried microspheres of batch # 2, in aqueous dispersion, was measured using a light scattering instrument. (Beckman Coulter, Model # LS 13320).

3) Preparation of nanospheres: For this study, 2 batches of nanospheres were prepared.

Batch # 1:

3 g of PLA is dissolved in 75 ml of chloroform. 195 mg of dexamathasone is added to the solution. The drug/polymer/solvent mixture is emulsified in 420 ml of 2% PVA solution. (The solution is prepared by dissolving 8.4 g PVA in 420 ml of water). The system is then sonicated using an ultra-sonicator (Misonix Incorporated; Sonicator Ultrasonic Processor XL) fitted with a micro-tip at a power of about 7 for 4 minutes. (Sonication is an act of applying sound energy to agitate particles in a sample for various purposes. It is applied using an ultrasonic probe colloquially known as a sonicator). It is used for breaking down and evenly dispersing the particles in the solution. After sonication, the system is mechanically agitated using an impeller (1/2" diameter) at 1000 rpm till the solvent evaporates. After all the solvent has evaporated, the nanopsheres are obtained by ultracentrifuging the suspension in an ultracentrifuge (DuPont Instruments-Sorvall; RC-5 Superspeed Refrigerated Centrifuge; Rotor # SS-34) at 7000 rpm for 10 minutes. The nanospheres are then lyophilized to obtain them in powder form.

Tableting:

The nanospheres of batch # 1 were then compressed into tablets using a compression press (Carver press; Model # 4350.L) and a custom made stainless steel mold with diameter of 8 mm. All the samples weighed about 200-220 mg. The nanospheres were compressed at 300,000 psi.

Batch # 2:

4 g of PLGA is dissolved in 100 ml of chloroform. 260 mg of dexamathasone is added to the solution. The drug/polymer/solvent mixture is emulsified in 500 ml of 2% PVA solution. (The solution is prepared by dissolving 10 g PVA in 500 ml of water). The system is then sonicated using an ultra-sonicator (Misonix Incorporated; Sonicator Ultrasonic Processor XL) with a micro-tip at a power of about 7 for 3 min. (Sonication is an act of applying sound energy to agitate particles in a sample for various purposes. It is applied using an ultrasonic probe colloquially known as a sonicator). It is used for breaking down and evenly dispersing the particles in the solution. After sonication, the system is mechanically agitated using an impeller (1" diameter) at 2000 rpm for 45 minutes and then at 1200 rpm till the solvent evaporates. After all the solvent has evaporated, the nanopsheres are obtained by ultracentrifuging the solution in an ultracentrifuge (DuPont Instruments-Sorvall; RC-5 Superspeed Refrigerated Centrifuge; Rotor # SS-34) at 7000 rpm for 10 minutes. The nanospheres are then lyophilized to obtain them in powder form.

Tableting:

The nanospheres of batch # 2 were then compressed into tablets using a compression press (Carver press; Model # 4350.L) and a custom made stainless steel mold with diameter of 8 mm. All the samples weighed about 200-220 mg. The nanospheres were compressed at 100,000 psi and 300,000 psi.

Particle size measurement:

The mean diameter of the freeze-dried nanospheres of batch # 2, in aqueous dispersion, was measured using a light scattering instrument. (Beckman Coulter, Model # LS 13320).

4) Drug release mechanisms:

The release mechanism for batch # 1 of microspheres and nanospheres is as follows:

The compressed tablet is placed in a conical tube and 5 ml of buffer (PBS) solution is added to it. The tube is then placed on a shaker (VWR Scientific Products; Orbital Shaker). At the pre-designated time point, the tube is removed from the shaker and centrifuged at 2000 rpm, for 5 min for the microspheres and 10 min for the nanopsheres. Approximately 4 ml of the supernatant is collected, after centrifugation, without disturbing the tablet. The supernatant is collected as the sample, in a glass tube. Approximately 4 ml of the buffer solution is replenished in the tube by tilting the tube at a 45 degrees angle and adding the buffer carefully to avoid disturbing the tablet. The tube is placed back on the shaker and the procedure is continued for every time point. The samples are read on a UV plate reader (Tecan, Infinite M200).

The release mechanism for batch # 2 of microspheres and nanospheres is as follows:

The compressed tablet is placed in a conical tube and 5 ml of buffer (PBS) solution is added to it. The tube is then placed on a rotating agitator (Barnstead Thermolyne; Labquake). At the pre-designated time point, the tube is removed from

the rotating agitator and centrifuged at 1200 rpm for 5 min for microspheres, and 2000 rpm for 5 min for the nanospheres. Approximately 4 ml of the supernatant is collected, after centrifugation, without disturbing the tablet. The supernatant is collected as the sample in a glass tube. Approximately 4 ml of buffer solution is replenished in the tube. After adding the buffer, the tablet is dispersed in the buffer by vortexing the tube on a vortexer for about a minute, till the tablet gets dispersed in the buffer. The tube is placed back on the rotating agitator. This procedure is continued and samples are collected at every time point. The samples are then read on a UV plate reader (Tecan, Infinite M200).

Results:

1) Particle size measurement:

a) Microspheres batch # 1: These microsphere tablets were compressed using 3 different compression pressures. The particle size of a sample tablet from every pressure was measured.

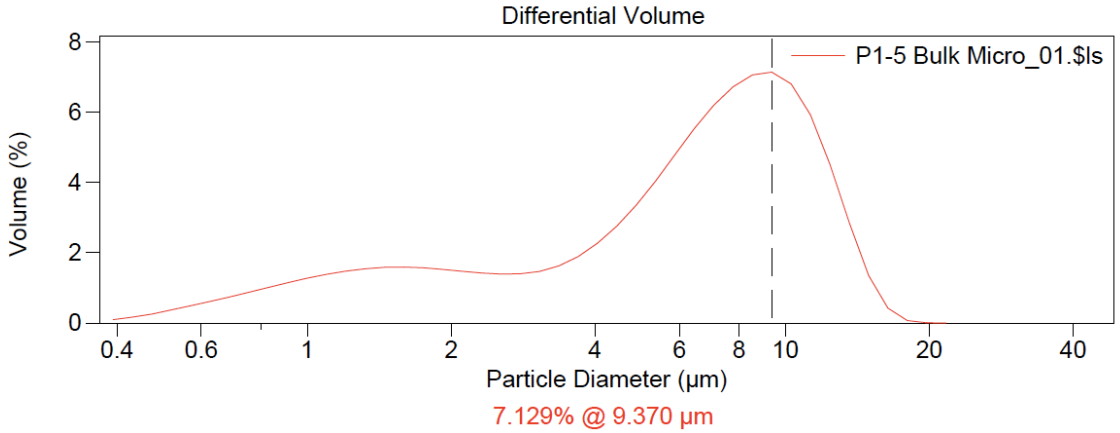


Fig. 7: Particle size curve for microspheres batch # 1 compressed at 100K psi

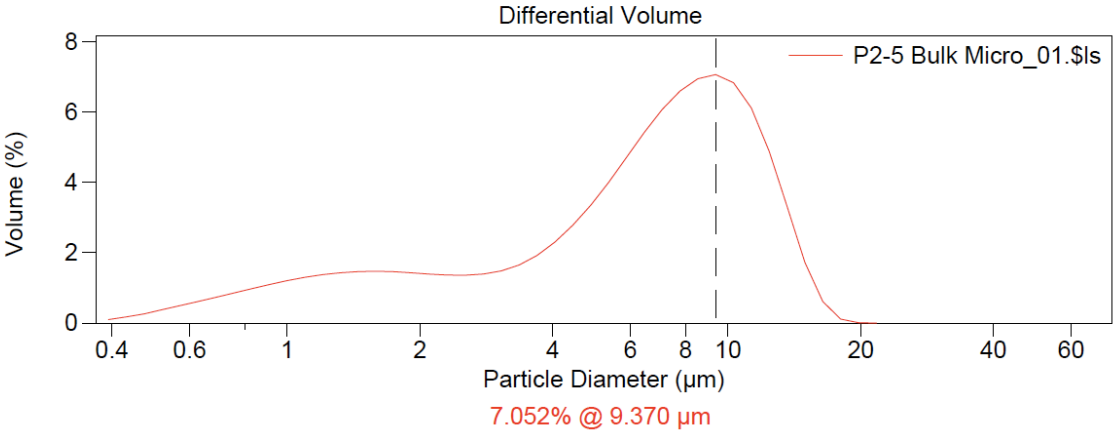


Fig. 8: Particle size curve for microspheres batch # 1 compressed at 200K psi

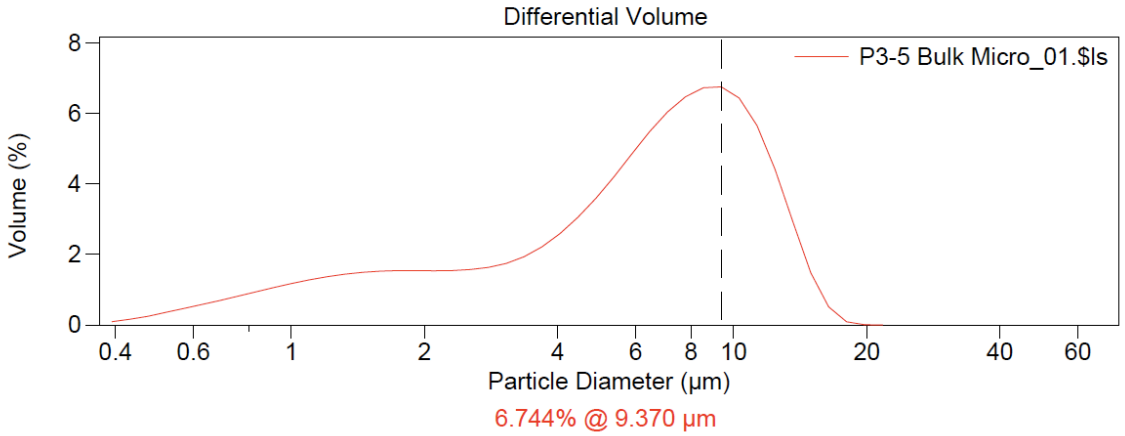


Fig. 9: Particle size curve for microspheres batch # 1 compressed at 300K psi

b) Microspheres batch # 2:

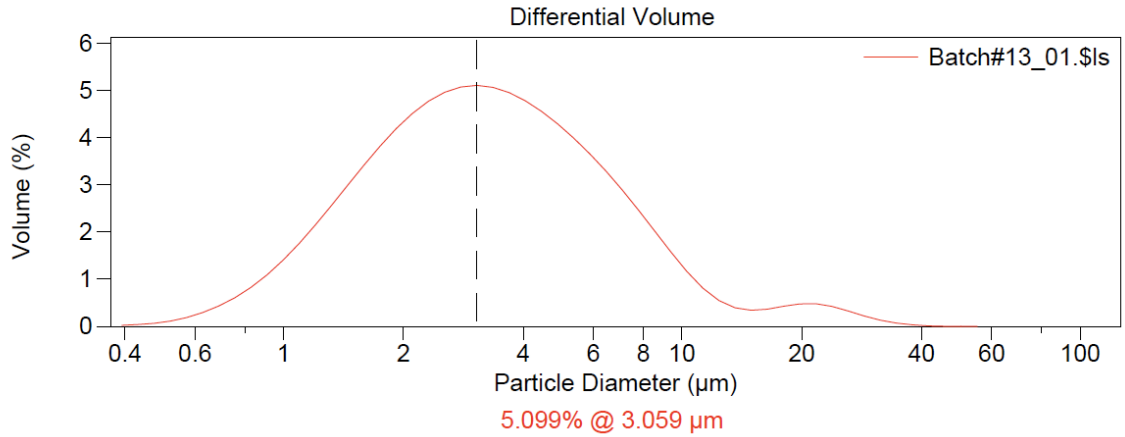


Fig. 10: Particle size curve for microspheres batch # 2

c) Nanospheres batch # 2

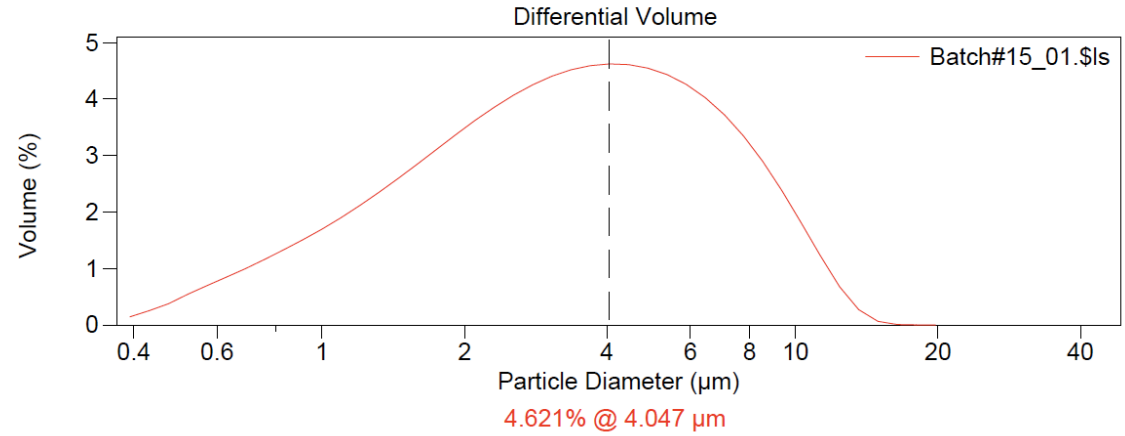


Fig. 11: Particle size curve for nanospheres batch # 2

2) Drug Release Mechanism:

The direct results obtained from this test were the absorbance values of the drug, measured from each sample. These absorbance values were then compared to a standard plot. Using this plot, they were converted from absorbance values to concentration units (micro gm/ml). Further calculation was performed to finally achieve net amount of drug released from each tablet. Cumulative values were obtained for increasing time points during the length of the study. Finally, average and standard deviation were calculated.

The result of the release study for each batch was plotted as a graph of:

i. Average of cumulative values Vs Time (with error bars)
ii. Average of cumulative values Vs Time (without error bars)
iii. Standard deviation of cumulative values Vs Time

1) Microspheres Batch # 1:
P1 = 100,000 psi
P2 = 200,000 psi
P3 = 300,000 psi

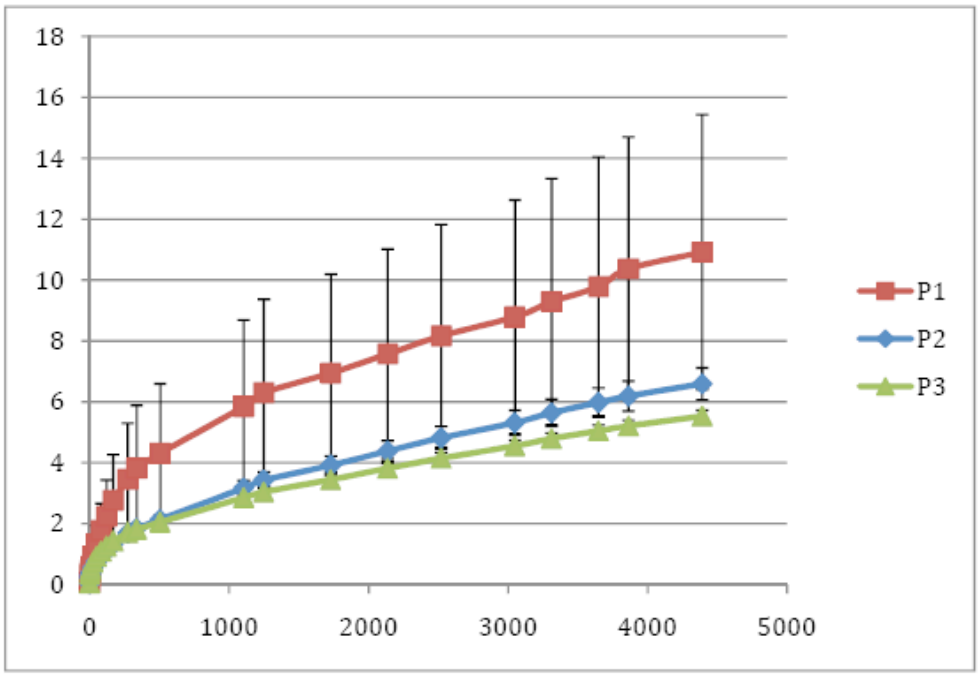


Fig. 12: Microspheres batch # 1: Cumulative average Vs Time (hours) with error bars

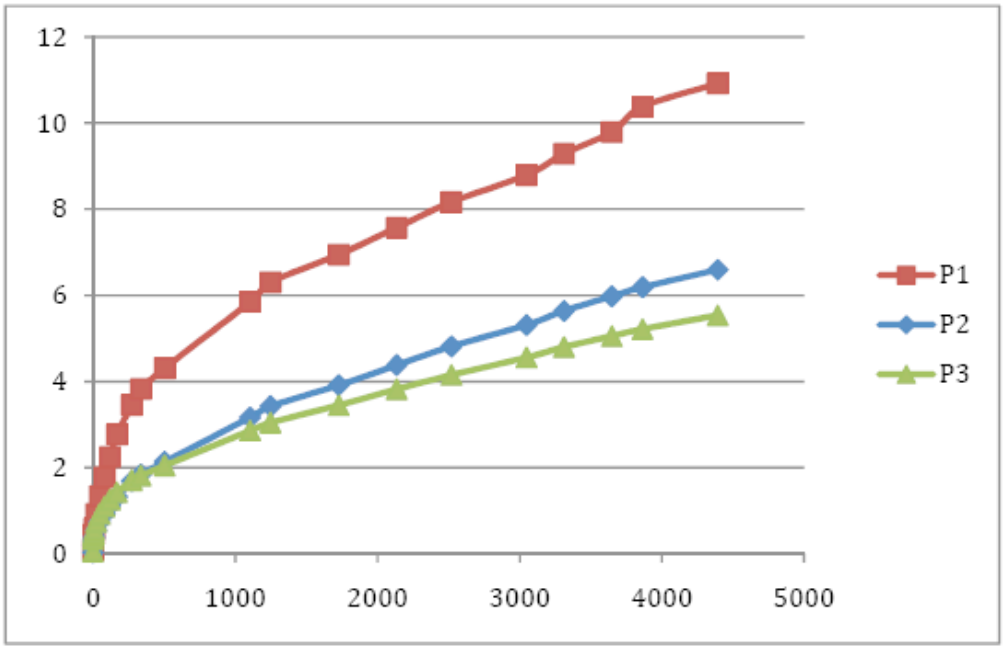


Fig. 13: Microspheres batch # 1: Cumulative average Vs Time (hours) without error bars

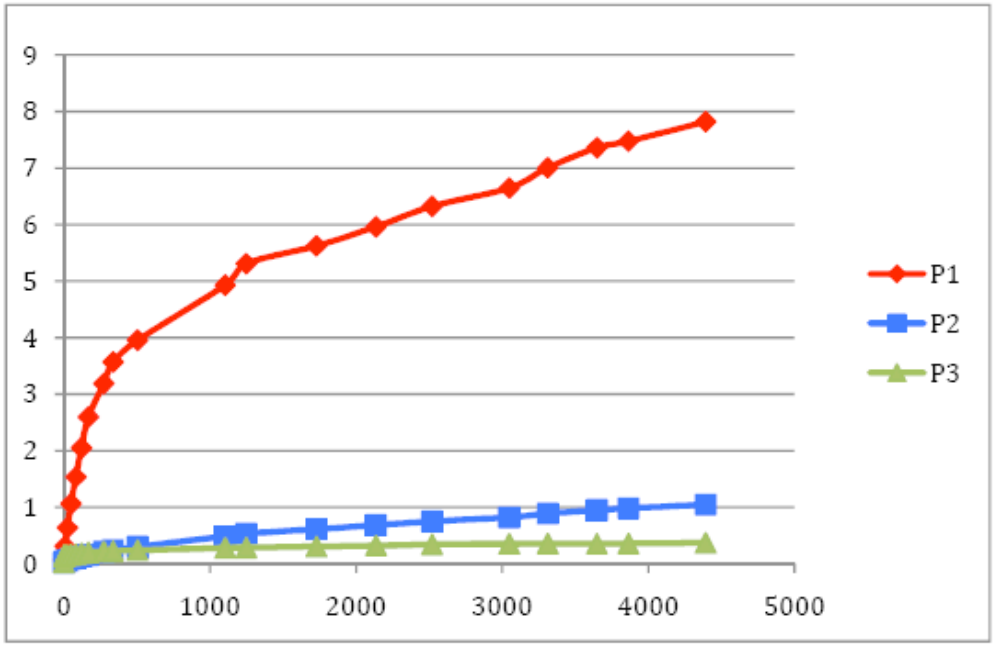


Fig. 14: Microspheres batch # 1: Cumulative standard deviation Vs Time (hours)

2) Microspheres Batch # 2:
   P1 = 100,000 psi
   P2 = 300,000 psi

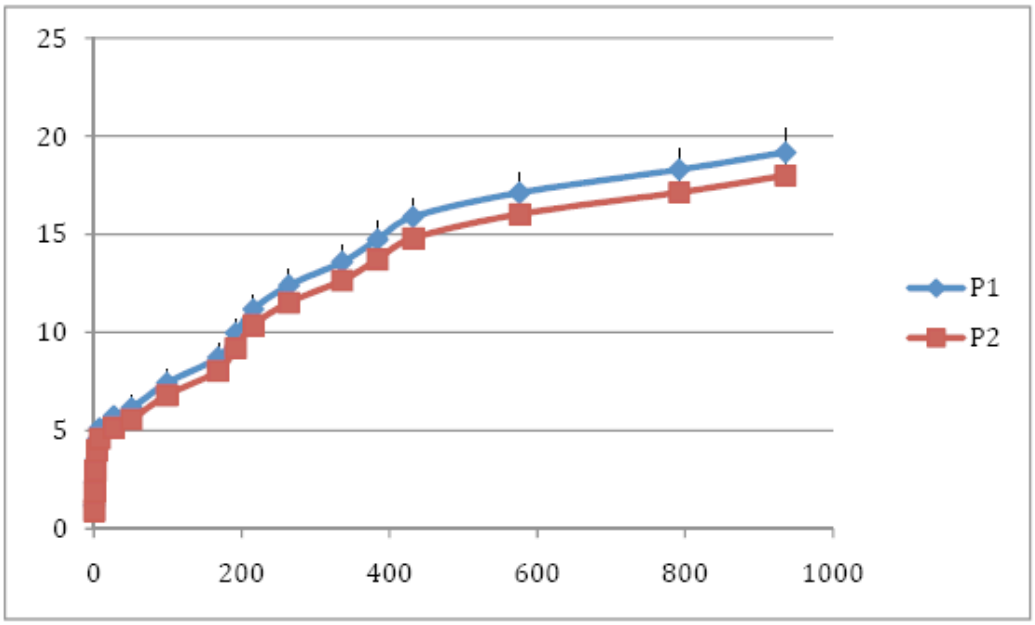


Fig. 15: Microspheres batch #2: Cumulative average Vs Time (hours) with error bars

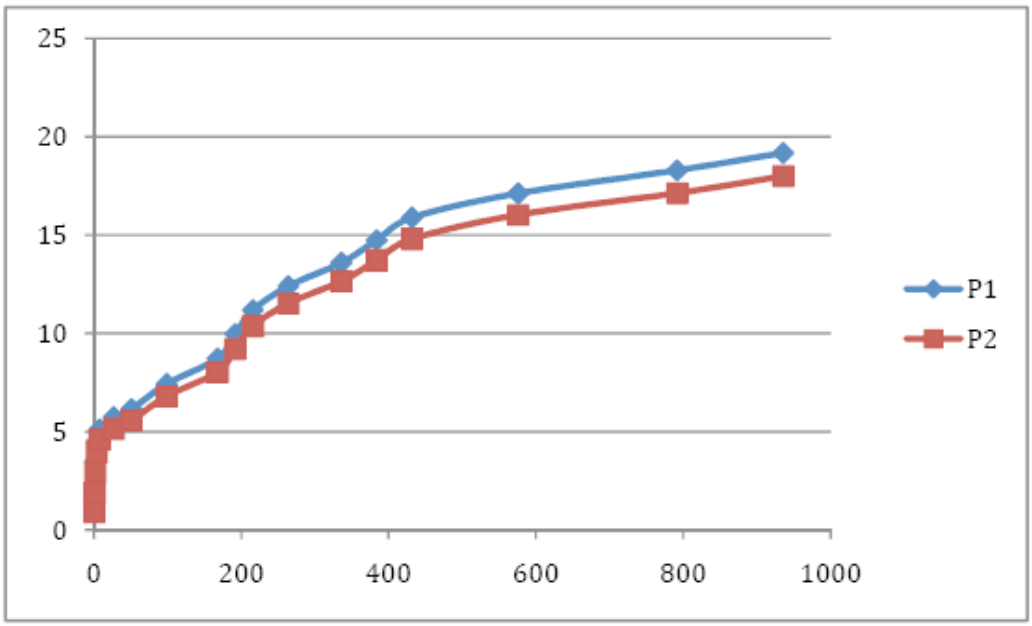


Fig. 16: Microspheres batch # 2: Cumulative average Vs Time (hours) without error bars

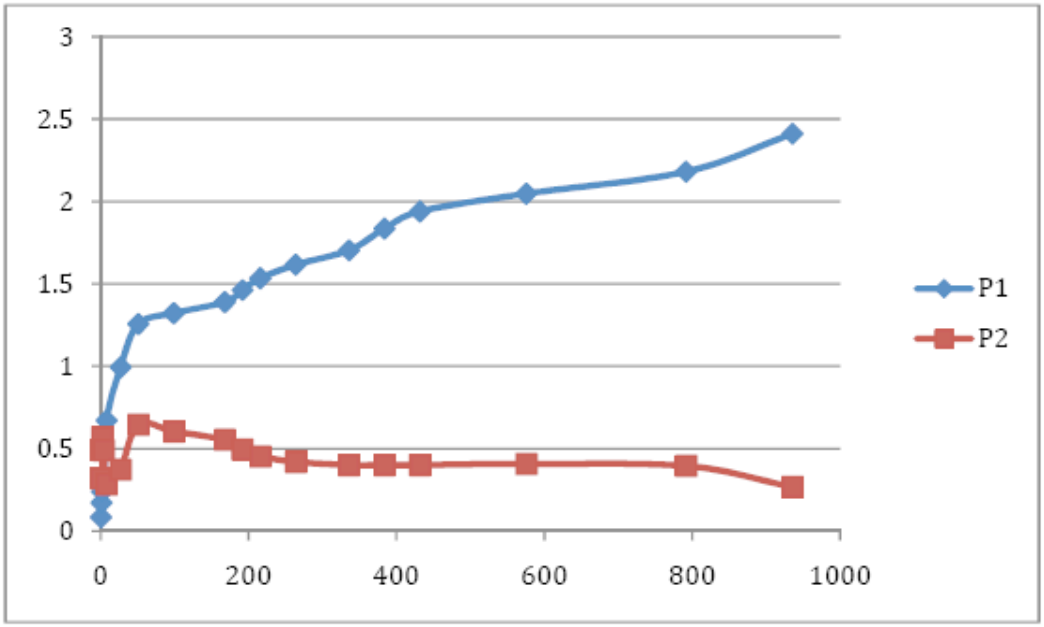


Fig. 17: Microspheres batch # 2: Cumulative standard deviation Vs Time (hours)

3) Nanospheres Batch # 1:
P = 300,000 psi

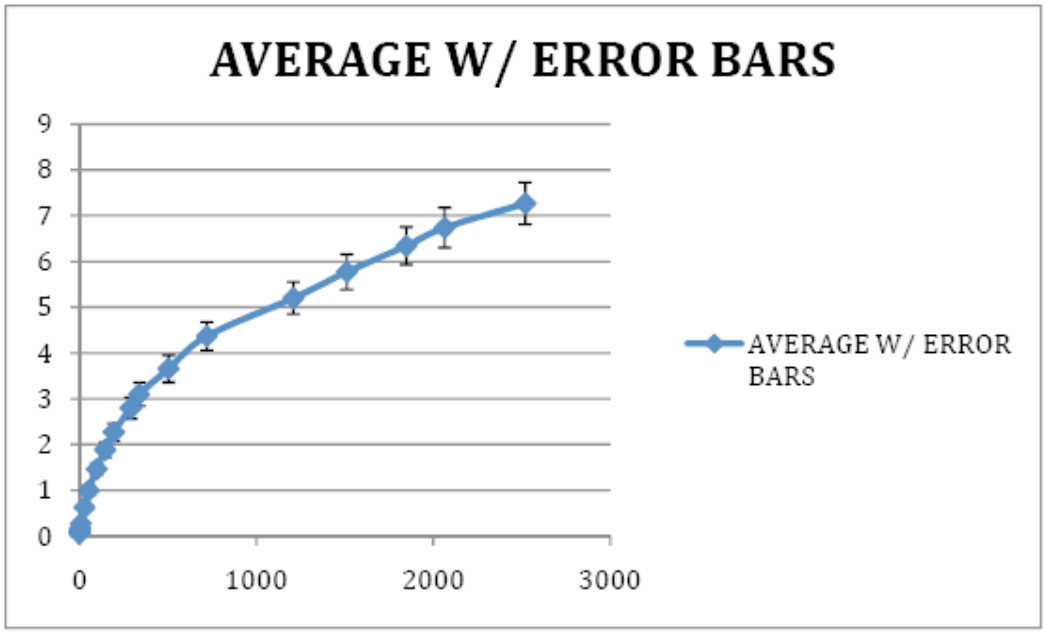


Fig. 18: Nanospheres batch # 1: Cumulative average Vs Time (hours) with error bars

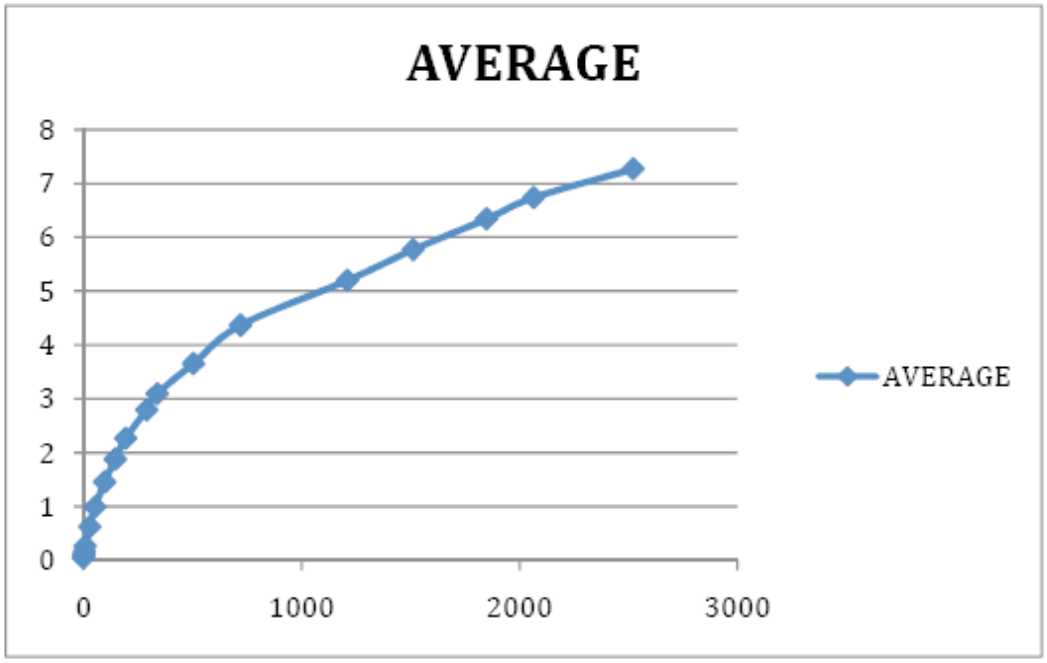


Fig. 19: Nanospheres batch # 1: Cumulative average Vs Time (hours) without error bars

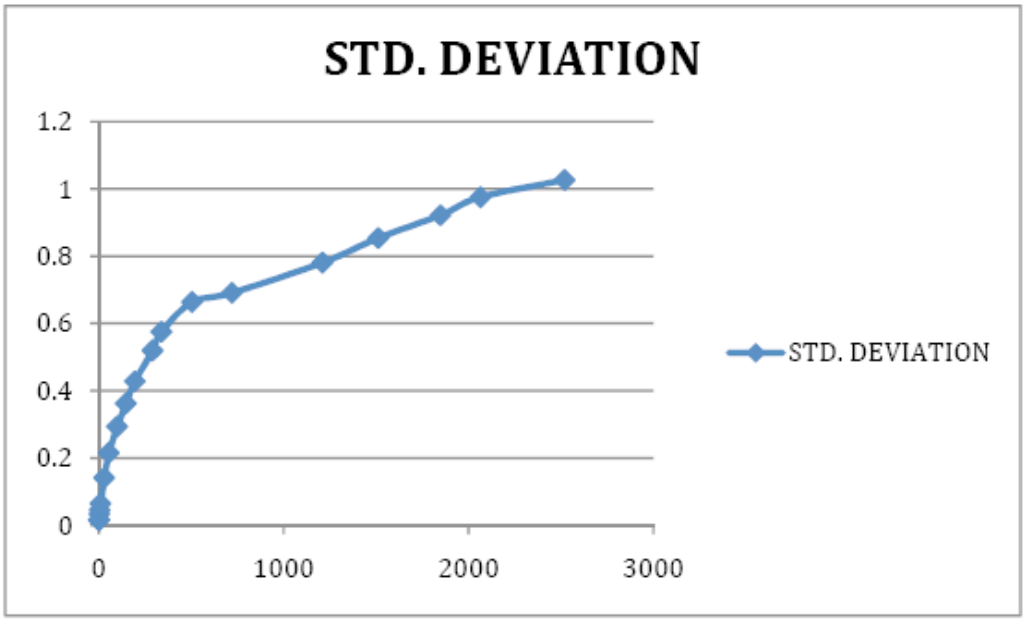


Fig. 20: Nanospheres batch # 1: Cumulative standard deviation Vs Time (hours)

4) Nanospheres Batch # 2:
   P1 = 100,000 psi
   P2 = 300,000 psi

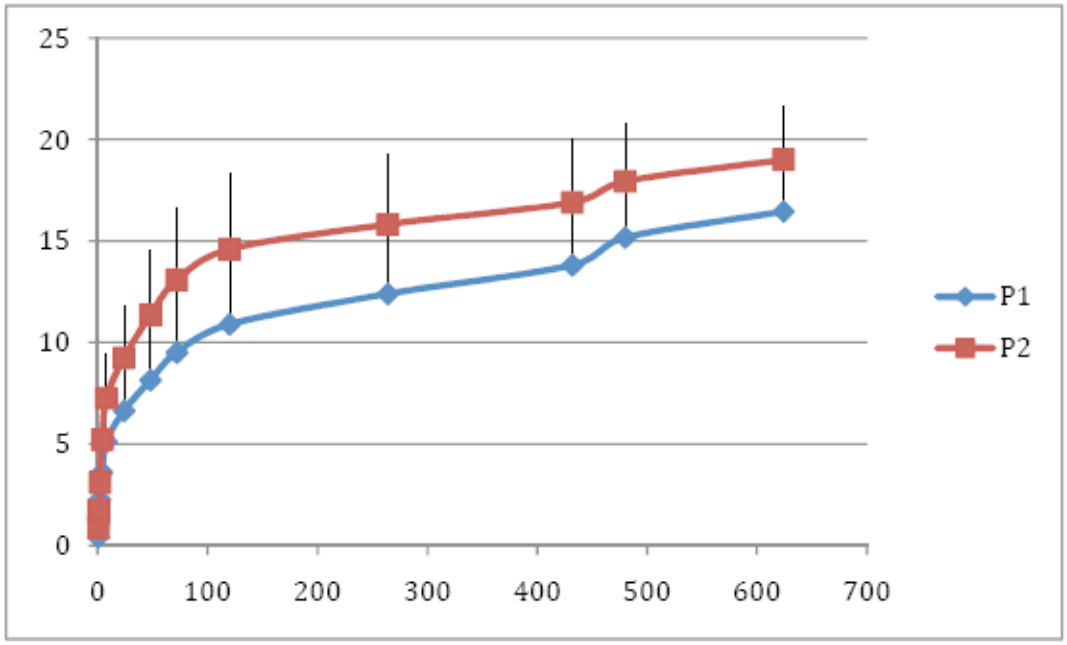


Fig. 21: Nanospheres batch # 2: Cumulative average Vs Time (hours) with error bars

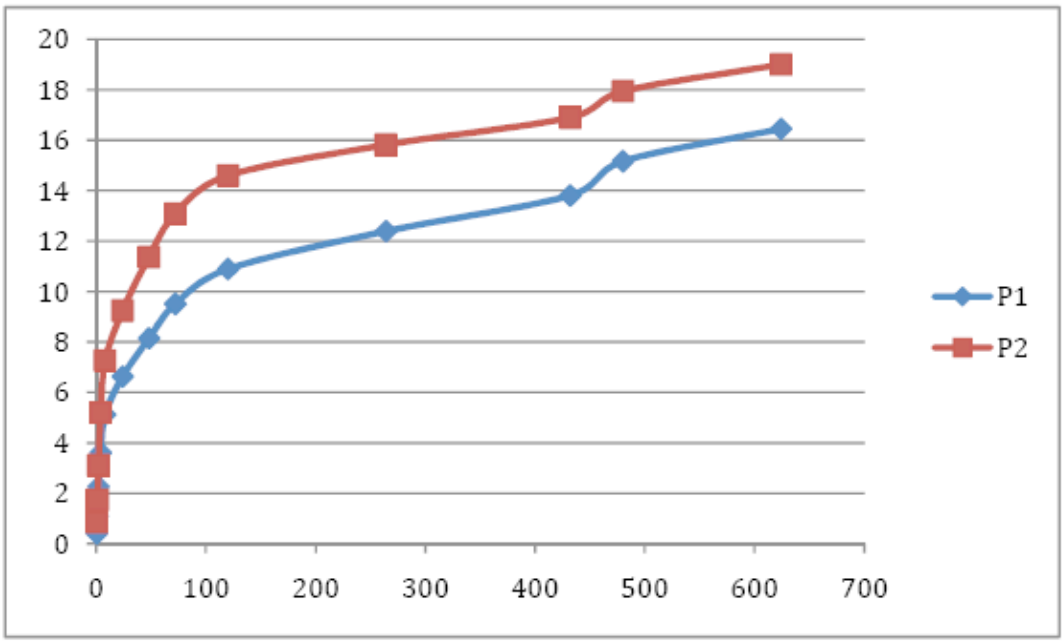


Fig. 22: Nanospheres batch #2: Cumulative average Vs Time (hours) without error bars

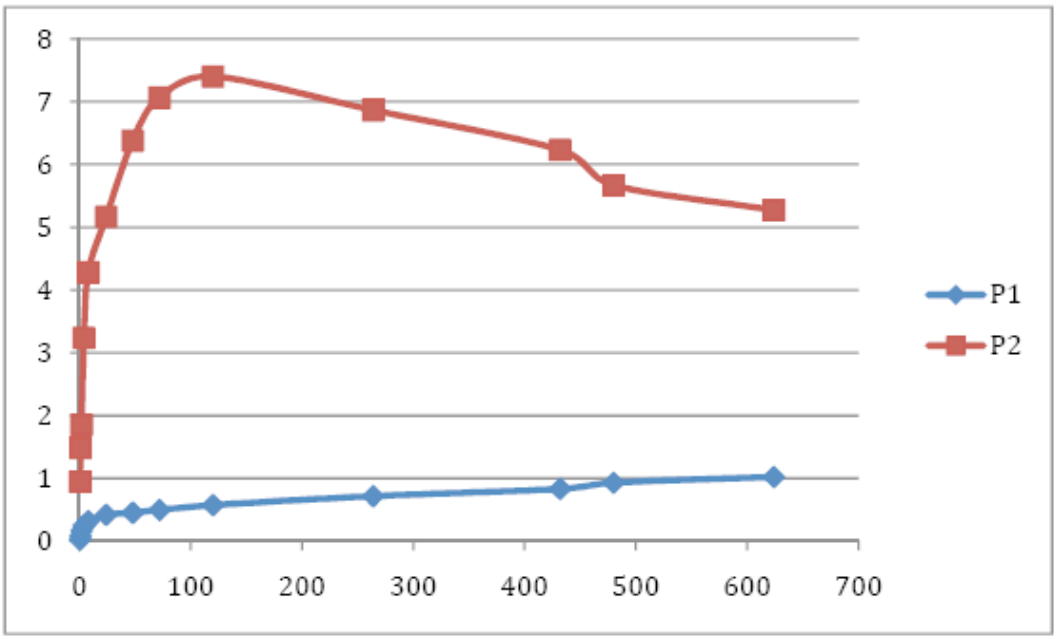


Fig. 23: Nanospheres batch # 2: Cumulative standard deviation Vs Time (hours)

Conclusions:

1) Particle size measurement:

   The particle size results of microsphere batch # 1 and batch # 2 conclude that the particles formed were indeed microspheres and were in the micron size range. Thus, the method of preparation of the microspheres seemed to be accurate and precise in forming the microspheres.

   The particle size results of nanospheres batch # 2 conclude that the particles formed, were in the low micron size range, rather than nano size range. Thus, the method of preparation needs to be modified, as indicated by the results, to assure formulation of particles in the nano size range.

2) Drug release study:

   The microspheres batch # 1 drug release study showed that tablets compressed at 100 K psi pressure release a larger amount of drug over a period of time as compared to those prepared by higher compression pressures. Also, there was a considerable deviation between the 3 samples of tablets compressed at 100 K psi as compared to their counterparts prepared by higher compression pressure. This could have been due to the difference in particle size of the particles compressed at 100K psi as compared to their counterparts prepared by higher compression pressure. The particles compressed at 100K psi could have been from the lower size range of the particle size curve. Thus, these particles released a larger amount of drug as compared to their counterparts from the higher size range of the particle size curve.

   The microspheres batch # 2 drug release study showed that the tablets compressed at different compression pressures release the drug following a similar trend. There was not much difference in the amount of drug released. The tablet samples compressed at 100 K psi show a small amount of deviation as compared to 300 K psi.

   The nanospheres batch # 1 drug release study showed a consistent increase in the release of the drug over a period of time. There was some deviation observed in the samples.

   The nanospheres batch # 2 drug release study showed that tablets compressed at 300 K psi release a larger amount of drug over the same period of time as compared to tablets compressed at 100 K psi. Also, tablets compressed at 300 K psi showed a huge amount of deviation among the different samples. This deviation was caused due to 1 tablet compressed at 300K psi, which released a much larger amount of drug as compared to its counterparts compressed at the same pressure. This could be due to the presence of lower particle size particles in that particular tablet which caused a much larger amount of drug release.

   This concludes that microspheres and nanospheres behave differently in terms of their release profile. Also, every sample undergoing the release study behaves in an individual manner following the general trend resulting in some deviation. Also, the drug release for microspheres and nanospheres is at a consistent increase over a period of time. This form of drug delivery is thus, very effective in volume sensitive applications in the human body.

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