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REVIEW ARTICLE

FROM FORMULATION VARIABLES TO DRUG ENTRAPMENT EFFICIENCY OF MICROSPHERES: A TECHNICAL REVIEW

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Received 18 April 2012; Review Completed 11 Nov 2012; Accepted 11 Nov 2012, Available online 15 Nov 2012

ABSTRACT

Purpose of writing this review on microspheres was to compile the recent literature with special focus on formulation variables which affect the drug entrapment efficiency of microspheres. There are various approaches in delivering a therapeutic substance to the target site in a controlled release fashion. One such approach is using microspheres as carriers for drugs. Microencapsulation is used to modify and delayed drug release form pharmaceutical dosage forms. For success of microspheres as drug delivery system its necessary to obtained desired particle size, maximum drug entrapment, mucoadhesion, swelling index and drug release. This can be obtained by optimizing the formulation as well as process variables but before designing the microspheres formulation deep understanding the effect of various variables on characteristics of microspheres is necessary. The intent of the paper is to highlight the reported study on various formulation variables those are might be useful to encountered several problems which is reason for low drug entrapment efficiency.

Key Words: Formulation variables, Microspheres, Controlled release, part, Drug entrapment, Mucoadhesion

INTRODUCTION:

Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as Microspheres[1], nanoparticles, liposomes, *etc.* which modulates the release and absorption characteristics of the drug. Dosage forms that can precisely control the release rates and target drugs to a specific body site have created enormous impact on the formulation and development of novel drug delivery systems [2]. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner [3, 4]. Microspheres constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteristics. Microspheres have many applications in medicine, with the main uses being for the encapsulation of drugs and proteins. Microparticulate systems can be made by various techniques involving physicochemical processes (solvent evaporation method, phase separation method) and mechanical processes (e.g., spray drying) [5].

A protein delivery system with high loading capacity is very advantageous, because it can prevent the loss of antigen and also limit the need of administering high level of carrier [6]. Several difficulties are faced in designing of microspheres better absorption and enhanced bioavailability. The formulation variables have a variety of effects on the physicochemical properties of the microspheres. The bio-distribution of the drug from microspheres is highly dependent on the size and % drug entrapment of the microspheres. Release kinetics of the microsphere matrix is depend on the various factors i.e. type of polymer used [1], concentration of polymer [1, 7-11], drug to polymer ratio, solubility of drug, dispersed phase to continuous phase ratio etc. These variables directly affect the loading efficiency of the microspheres. In solvent evaporation method entrapment efficiency of water-soluble drugs is low due to drug loss from the organic emulsified polymeric phase before solidification of polymer in the microspheres [12, 13]. Therefore, process optimization and formulation optimization are advantageous for the efficient entrapment of water-soluble labile drugs like therapeutic enzymes. Optimum formulation can be made possible by understanding of variables which affect the particle size, drug entrapment, swelling index, mucoadhesion and drug release of microspheres. Purpose of writing this review was to compile the recent literature on the various formulation variables influencing the characteristics of microspheres. Additionally this also summarized the method of preparation and characterization of microspheres.

FACTORS INFLUENCING DRUG ENTRAPMENT EFFICIENCY OF MICROSPHERES:

Deep understanding of effects of some important factors and their interactions during the process of preparation on Microparticles physicochemical properties are necessary before designing and evaluation of microspheres.

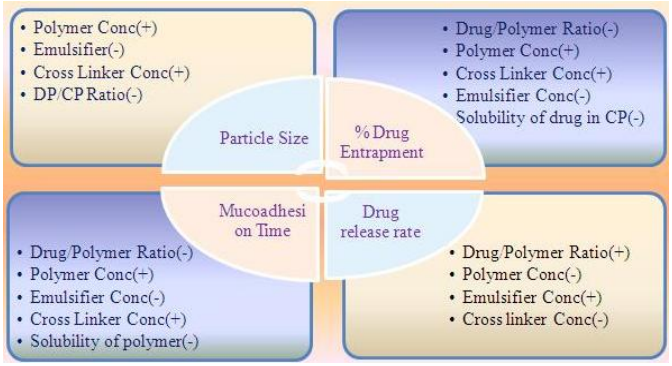

Fig 1: Formulation variables and their effect on microspheres (+→ Increase, — →Decrease)

Concentration of the polymer in dispersed phase:

Results from different study shows that the particle size, swelling, loading efficiency and rate of drug release from the microspheres depended on the polymer concentration and the type of polymer used.

Encapsulation efficiency increases with increasing polymer concentration [7-9]. For example, the encapsulation efficiency increased from 53.1 to 70.9% when concentration of the polymer increased from 20.0 to 32.5% [7]. High viscosity and fast solidification of the dispersed phase contributed to reduce porosity of the microparticles as well [10]. The contribution of a high polymer concentration to the loading efficiency can be interpreted in three ways. First, when highly concentrated, the polymer precipitates faster on the surface of the dispersed phase and prevents drug diffusion across the phase boundary [8]. Second, the high concentration increases viscosity of the solution and delays the drug diffusion within the polymer droplets [11]. Third, the high polymer concentration results large size of microspheres which result in loss of drug from surface during washing of microspheres is very less as compare to small microspheres. Thus size of microspheres is also affecting the loading efficiency [1].

X. Fu et al., studied the effect of molecular weight of the polymer on encapsulation efficiency, developed a long-acting injectable huperzine A-PLGA microsphere for the chronic therapy of Alzheimer's disease, the microsphere was prepared by using o/w emulsion solvent extraction evaporation method. The encapsulation efficiency of the microspheres improved as the polymer concentration increase in oil phase and PVA concentration decreased in aqueous phase. [15]

Thakkar et al investigated the effect of polymer concentration on the encapsulation efficiency of the Celecoxib Microspheres of natural polymer (bovine serum albumin) BSA using emulsification chemical cross-linking method. Results from this investigation shows that increase in concentration of BSA significantly increase the encapsulation efficiency of microspheres. The entrapment efficiency increases with an increase in the albumin concentration because with an increase in the albumin concentration, more viscous solutions are formed that can more efficiently prevent the dissolution of Celecoxib in the external phase of the emulsion. At a lower concentration of albumin, a major amount of the drug remained as free drug [16].

Agrawal et al studied the effects of variables such as polymer concentration on the particle size, drug release and loading efficiency of microspheres at increasing Polymer concentrations (*i.e.*, at drug–Polymer ratios from 1:2 to 1:6) increased from 135.3 to 163.4 mm. This increase in particle size of the microspheres can be attributed to an increase in viscosity with increasing polymer concentrations, which resulted in larger emulsion droplets and finally in greater microsphere Size. The release of albendazole from microspheres decreased as the Polymer concentration increased, suggesting that drug release could be controlled by varying the Polymer concentration. The results might also be explained by the fact that the higher Polymer content resulted in larger particles with proportionately less drug, so that the drug–polymer ratio was changed and thus release was reduced. [17]

Another study shown that increase of mean particle size with increase in polymer concentration may have occurred due to the fact that as polymer concentration increases it produces a significant increase in the viscosity in a fixed volume of solvent, thus leading to an increase of the emulsion droplet size and finally a higher microsphere size. [15, 17-27]

The drug entrapment efficiency of microspheres was also improved with changing the concentration of drug and polymer in the internal phase to the higher concentration. This may be due to the increase in the viscosity of the internal phase that reduces the migration of the drug molecules in the aqueous phase.[28]

Results from study by Lakshmana Prabu S et al revealed that the drug content of microspheres was not affected by the volume of dichloromethane, but the particle sizes were found to change significantly. This may also be due to the increase in the volume of dichloromethane leads to decrease in viscosity of the internal phase could be an effective factor in the droplet size of the emulsion in the aqueous medium. In this case, it seems that the shear effect of the propeller is able to break the large droplets into smaller ones, which are solidified into microspheres on solvent evaporation. [31]

Drug: Polymer Ratio (DPR):

The drug entrapment efficiency within microspheres produced using the solvent evaporation method is of fundamental importance as failure to achieve acceptable drug loadings may preclude the use of this method for economic reasons [32] Trivedi et al prepared Aceclofenac microspheres by emulsion-solvent evaporation method using Eudragit RL100, Eudragit RS100 and Eudragit S100. Results from this study clearly indicate that encapsulation efficiency is significantly increasing as the DPR decreased [33]. Nagda et al reported that encapsulation efficiency of carbopol microspheres significantly increase as the amount of polymer is increased at the same amount of drug in the dispersed [34].

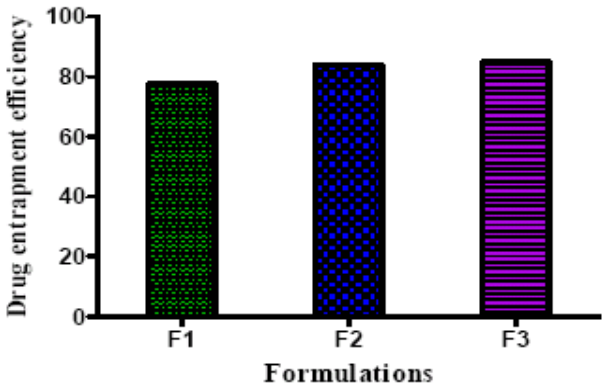

Fig 2: Drug entrapment efficiency of Trimetazidine hydrochloride Microspheres [34]

Pavanveena et al prepared trimetazidine hydrochloride loaded chitosan microspheres and studied the effect of drug: polymer ratio on the loading efficiency of these microspheres. Three different formulations with drug: polymer ratios (1:1, 1:2, 1:3) are prepared and coded as F1, F2 and F3. Figure 2 shows increase the loading efficiency as increase in amount polymer while drug content keeping constant [35].

Drug release from microspheres is notably affected by the ratio of the drug to the polymer as increasing in the first causes faster drug release. By increasing the amount of drug loading, a point will be reached when the solid drug particles upon dissolution will begin to form continuous pores or channels within the matrix. Under these circumstances, the path of release for drug molecules will be diffusion within the channels formed from areas where drug has previously leached out from the matrix [36, 37]. In other words, as the amount of drug content is increased the matrix will become more porous as drug is leached out from the polymer and thus faster drug release rate occurs [38].

Solubility of polymer in the solvent:

Mehta et al.[7], studied the effect of solubility of different PLGAs polymers in methylene chloride were compared by measuring the methanol cloud point (Cs): Higher Cs meant that the polymer was more soluble in methylene chloride and, thus, required a greater amount of methanol to precipitate from the polymer solution. The PLGA polymer of a relatively high L/G ratio (75/25) had a higher solubility in methylene chloride than the other PLGA (L/G ratio=50/50). A lower molecular weight polymer had a higher solubility in methylene chloride than a higher molecular weight polymer. End-capped polymers, which were more hydrophobic than non-end-capped polymers of the same molecular weight and component ratio, were more soluble in methylene chloride. Diffusion of drugs into the continuous phase mostly occurred during the first 10 minutes of emulsification; therefore, as the time the polymer phase stayed in the non-solidified (semi-solid) state was extended, encapsulation efficiency became relatively low. In Mehta's study, polymers having relatively high solubility in methylene chloride took longer to solidify and resulted in low encapsulation efficiencies, and vice versa[7]. Particle size and bulk density also varied according to the polymer. Since polymers having higher solubility in methylene chloride stayed longer in the semi-solid state, the dispersed phase became more concentrated before it completely solidified, resulting in denser microparticles.

Selection of solvent system for the dispersed phase

Selection of solvent system based on the volatility of solvent and solubility of polymer and type of method of preparation used for preparation of microspheres. Solvent should have high volatility and high polymer solubility. Jia Yu et al were used mixture of methanol and methylene chloride (1: 9) as the organic phase to increase the solubility of the drug. In this process, an increase was observed in the rate of precipitation of the polymer in the droplet–water interface; thus, the loss of drug into the outer aqueous phase was minimized, resulting in homogeneous and smaller particles [39].

Bodmeier et al found that methylene chloride resulted in higher encapsulation efficiency as compared with chloroform or benzene, even though methylene chloride was a better solvent for poly (lactic acid) (PLA) than the others. Methylene chloride is more soluble in water than chloroform or benzene. The 'high' solubility allowed relatively fast mass-transfer between the dispersed and the continuous phases and led to fast precipitation of the polymer. The significance of solubility of the organic solvent in water was also confirmed by the fact that the addition of water-miscible co-solvents such as acetone, methanol, ethyl acetate, or dimethyl sulfoxide (DMSO), contributed to increase of the encapsulation efficiency [11]. Knowing that the methanol is a non-solvent for PLA and a water-miscible solvent, it can be assumed that methanol played a dual function in facilitating the polymer precipitation: First, the presence of methanol in the dispersed phase decreased the polymer solubility in the dispersed phase [40]. Second, as a water-miscible solvent, methanol facilitated diffusion of water into the dispersed phase.

Park et al. were prepared lysozyme-loaded PLGA microparticles using the oil in water (o/w) single emulsion technique. Here, the authors used a co-solvent system, varying the ratio of the component solvents. DMSO was used for solubilization of lysozyme and PLGA, and methylene chloride was used for generation of emulsion drops as well as solubilization of PLGA. Encapsulation efficiency increased, and initial burst decreased as the volume fraction of DMSO in the co-solvent system increased. Particle size increased, and density of the microparticle matrix decreased with increasing DMSO. Overall, these results indicate that the presence of DMSO increased the hydrophilicity of the solvent system and allowed fast extraction of the solvent into the continuous phase, which led to higher encapsulation efficiency and larger particle size [41].

Ratio of dispersed phase to continuous phase (D/C ratio):

No significant difference in particle size was observed ($P > 0.05$). All microspheres have a spherical shape without pores on the surface, with size approximately 20 µm. However, the drug loading and encapsulation efficiency

increased remarkably with decreasing D/C ratio ($P < 0.05$) [42]. Similar phenomena were reported for the encapsulation of progesterone [43]. Additionally, the surface of microspheres was smoother at lower D/C ratios, probably due to the faster solidification rate. It has been reported that the porosity in a system of microspheres is determined during microspheres hardening as the organic solvent evaporates during preparation [43]. Continuous phase containing a large amount of water resulted in faster polymer precipitation and therefore less porous spheres were formed [44].

Encapsulation efficiency and particle size increase as the volume of the continuous phase increases in case of O/W emulsification method. For example, the encapsulation efficiency increased more than twice as the ratio of the dispersed phase to the continuous phase (DP/CP ratio) decreased from 1/50 to 1/300[7-9]. It is likely that a large volume of continuous phase provides a high concentration gradient of the organic solvent across the phase boundary by diluting the solvent, leading to fast solidification of the microparticles. Sah et al utilized ethyl acetate as a solvent in polymer solution for the formation of microparticles When 8 mL of PLGA solution (o) was poured into 50 mL of water phase (w), the polymer solution was well disintegrated into dispersed droplets. On the other hand, when the continuous phase was 80 mL or more, the microspheres hardened quickly and formed irregular precipitates. This is because the large volume of continuous phase provided nearly a sink condition for ethyl acetate and extracted the solvent instantly. Due to the fast solidification of the polymer, particle size increased with increasing volume of the continuous phase [45]

As volume of continuous phase is increased, the size of microspheres decreased which results in decrease in loading efficiency, less mucoadhesion time and faster drug release. [14]

Interaction between drug and polymer:

Interaction between protein and polymer contributes to increasing encapsulation efficiency [46]. Generally, proteins are capable of ionic interactions and are better encapsulated within polymers that carry free carboxylic end groups than the end-capped polymers. On the other hand, if hydrophobic interaction is a dominant force between the protein and the polymer, relatively hydrophobic end-capped polymers are more advantageous in increasing encapsulation efficiency [7]. In certain cases, a co-encapsulated excipient can mediate the interaction between protein and polymer [47]. For example; encapsulation efficiency increased when gamma hydroxypropyl cyclodextrin (g-HPCD) were co-encapsulated with tetanus toxoid in PLGA microparticles. It is supposed that the g-HPCD increased the interaction by accommodating amino acid side groups of the toxoid into its cavity and simultaneously interacting with PLGA through Van-der Waals and hydrogen bonding forces.

Solubility of drug in continuous phase:

If the drug is more soluble in continuous phase, more drug loss in the continuous phase is occurs due to diffusion of drug from dispersed phase to continuous phase. If the solubility of the drug in the continuous phase is higher than in the dispersed phase, the drug will easily diffuse into the continuous phase during this stage which tends to decrease the encapsulation efficiency. For example, the encapsulation efficiency of quinidine sulfate was 40 times higher in the alkaline continuous phase (pH 12, in which quinidine sulfate is insoluble) than in the neutral continuous phase (pH 7, in which quinidine sulfate is very soluble) [11].

Effect of concentration of emulsifier:

Thakkar et al investigated the effect of emulsifier on the size, encapsulation efficiency and drug entrapment of the microspheres prepared using a natural polymer (bovine serum albumin) BSA using emulsification chemical cross-linking method. Results from this investigation shows that increase in concentration of Span-85 decrease the encapsulation efficiency of microspheres in some extent. This is due to fact that increase in Span-85 concentration leads to stabilization of small droplets and results in smaller microspheres. Loss of drug from surface of small microspheres is more as compared to larger microspheres during washing [16].

Rawat et al studied the Influence of Selected Formulation Variables on the Preparation of Enzyme-entrapped Eudragit S100 Microspheres. Figure 4 represent the response surface plot, which shows the effects of the DCM and Tween80 on the drug loading of microspheres. Drug loading decreased as the concentration of DCM was increased [19].

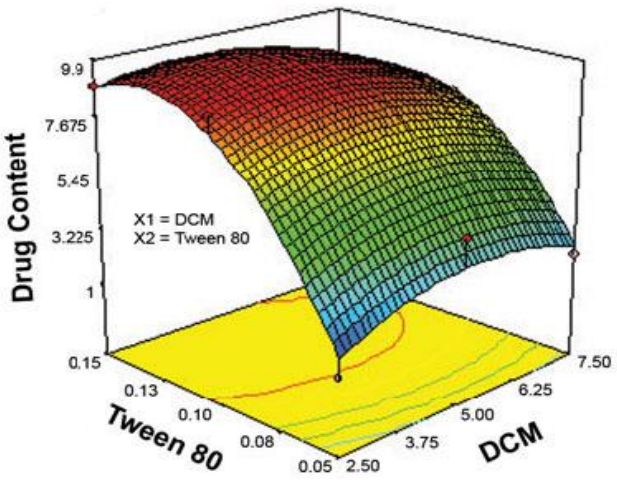

Fig 3: Effect of emulsifier (Tween 80) on the drug content of Microspheres [19]

Lakshmana Prabu S et al concluded that, amount of PVA as an emulsifying agent did not influence the drug loading and entrapment efficiency of microspheres however the particle size of microspheres is seen to be dependent on the PVA concentration in the continuous phase. The results revealed that on increasing PVA concentration, more PVA molecules may overlay the surface of the droplets, providing an increased protection of the droplets against coalescence resulting in the production of small emulsion droplets. Since microspheres were formed from emulsion droplets after solvent evaporation, their size was dependent on the size of emulsion droplets. [31]

Effect concentration of cross linking agent:

Patel et al has studied effect of cross linking agent on loading efficiency of mucoadhesive microspheres of glipzide. Result from this study showed significant effect on the percentage mucoadhesion and drug entrapment efficiency of microspheres. The higher amount of glutaraldehyde appears to favor the cross-linking reaction, and hence spherical free-flowing microspheres were obtained with an increase in loading efficiency [21].

CONCLUSION:

The purpose of this work was to understanding effect of various process as well as formulation variables on the encapsulation efficiency of the microspheres. This review will focus on how the formulation variables of microspheres formulation affect the drug entrapment efficiency the microspheres. This paper also explains that how drug entrapment efficiency depend upon particle size, Polymer concentration, type of polymer, drug: polymer ratio, DP: CP ratio, drug: polymer interaction, solubility of polymer as well as drug, method of preparation etc. The stirring rate of emulsion system, concentration of polymer, drug: polymer interactions, concentration of cross linkers are directly proportional to drug entrapment efficiency. Whereas higher drug to polymer ration, high concentration of emulsifier decrease the drug loading efficiency of microspheres. It is the reliable means to increase the loading efficiency, if optimize the formulation as well as process variables. This will only possible by understanding the effect of various variables which affect the drug entrapment efficiency of these microspheres. Among all the variables stirring speed, polymer concentration, solubility of drug and polymer and drug: polymer interactions are the variables which have significant effect on the drug entrapment efficiency.

ACKNOWLEDGEMENT:

Author is highly thankful to Dept of pharmacy, IEC Group of Institution Greater Noida (India) for providing him best facilities and good atmosphere for furnishing this work.

DECLARATION OF INTEREST: There are no declarations of interest.

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 ISSN: 2250-1177 CODEN (USA): JDDTAO