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Variables Influencing the Drug Entrapment Efficiency of Microspheres: A Pharmaceutical Review

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ABSTRACT

Novel drug delivery systems have several advantages over conventional multi dose therapy. Much research effort in developing novel drug delivery system has been focused on controlled release and sustained release dosage forms. Now considerable efforts are being made to deliver the drug in such a manner so as to get optimum benefits. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumor. Microencapsulation is used to modify and delayed drug release form pharmaceutical dosage forms. Microspheres efficiently utilized in controlled delivery of many drugs but wastage of drug due to low drug entrapment efficiency is the major drawback of such microparticulate system. Well designed microspheres can overcome such problems by enhancing the loading efficiency of a particular drug and minimizing the wastage of drug. 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. The intent of the paper is to highlight the various variables which influence the drug entrapment efficiency along with method of preparation and characterization of microspheres.

Key Words: Novel drug delivery system, Controlled release, Microspheres, Drug entrapment, formulation variables, process variables.

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. The drug loaded microspheres are delivered to the target area by passive means (trapping by size) or active means (magnetic targeting)[5] and slowly release the encapsulated drug over a desired time period, the length of which is determined by the drug's biological half-life and release kinetics of the microsphere matrix. 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, 6-10], drug to polymer ratio, solubility of drug, dispersed phase to continuous phase ratio etc. These variables directly affect the loading efficiency of the microspheres. Polymeric microspheres and microcapsules have received much attention for the delivery of therapeutically useful proteins in a controlled way[11] Microparticulate systems can be made by various techniques involving physicochemical processes (solvent evaporation method, phase separation method) and mechanical processes (e.g., spray drying)[12].

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 [13].
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 [14, 15]. Therefore, process optimization may be advantageous for the efficient entrapment of water-soluble labile drugs like therapeutic enzymes.

However, the success of microspheres is limited due low drug entrapment efficiency. Therefore process optimization by understanding of variables which affect the drug entrapment is very important for improving the loading efficiency of microspheres. Purpose of writing this review was to compile the recent literature which focus on the various variables influencing the drug loading efficiency and approaches to improve the loafing efficiency of microspheres. Additionally this also summarized the method of preparation and characterization of microspheres.

Preparation of microspheres:
The most commonly investigated techniques to prepare microspheres are emulsion solvent evaporation techniques, Spray drying, emulsion cross-linking method Solvent evaporation, Hot melt microencapsulation, Solvent removal, Hydrogel microspheres and Phase inversion Microencapsulation.

1. **Emulsion cross-linking method[16]**:
The drug was dissolved in an aqueous gelatin solution (10% w/v), which was preheated at 40° for 1 h. The solution was added drop wise to liquid paraffin while stirring the mixture at 1500 rpm at 35° for 10 m. This gives water in oil (W/O) emulsion. Stirring was continued for further 10 m at 15° and the microspheres were washed three times with acetone and isopropyl alcohol, respectively. The washed microspheres were air dried and then dispersed in 5 ml of aqueous glutaraldehyde-saturated toluene solution (25% v/v) at room temperature for 3 h to allow cross linking. The microspheres were washed with toluene and treated with 100 ml of 10 mM glycine solution containing 0.1% w/v Tween 80 at 37° for 10 m to block unreacted glutaraldehyde. The resultant microspheres were finally freeze-dried.
2. **Solvent Evaporation[17]:**
It is the most extensively used method of microencapsulation, first described by Ogawa *et al.* A buffered or plain aqueous solution of the drug (may contain a viscosity building or stabilizing agent) is added to an organic phase consisting of the polymer solution in solvents like dichloromethane (or ethyl acetate or chloroform) with vigorous stirring to form the primary water in oil emulsion. This emulsion is then added to a large volume of water containing an emulsifier like PVA or PVP to form the multiple emulsions (w/o/w). The double emulsion, so formed, is then subjected to stirring until most of the organic solvent evaporates, leaving solid microspheres. The microspheres can then be washed, centrifuged and lyophilize to obtain the free flowing and dried microspheres.
3. **Hot Melt Microencapsulation[18]:**
This method was first used by Mathiowitz and Langer to prepare microspheres of polyanhydride copolymer of poly [bis(*p*-carboxy phenoxy) propane anhydride] with sebacic acid. In this method, the polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50m m. The mixture is suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5° above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, *e.g.* polyanhydrides. Microspheres with diameter of 1—1000m m can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disadvantage of this method is moderate temperature to which the drug is exposed.
4. **Solvent Removal[19]:**
It is a non-aqueous method of microencapsulation, particularly suitable for water labile polymers such as the polyanhydrides. In this method, drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride. This mixture is then suspended in silicone oil containing span 85 and methylene chloride. After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting microspheres can then be dried in vacuum.
5. **Hydrogel Microspheres[20]:**
Microspheres made of gel-type polymers, such as alginate, are produced by dissolving the polymer in an aqueous solution, suspending the active ingredient in the mixture and extruding through a precision device, producing micro droplets which fall into a hardening bath that is slowly stirred. The hardening bath usually contains calcium chloride solution, whereby the divalent calcium ions crosslink the polymer forming gelled microspheres. The method involves an “all-aqueous” system and avoids residual solvents in microspheres. Lim and Moss developed this method for encapsulation of live cells, as it does not involve harsh conditions, which could kill the cells. The surface of these microspheres can be further

modified by coating them with polycationic polymers, like polylysine after fabrication. The particle size of microspheres can be controlled by using various size extruders or by varying the polymer solution flow rates.

6. **Spray Drying[21]:**
   In this process, the drug may be dissolved or dispersed in the polymer solution and spray dried. The quality of spray-dried microspheres can be improved by the addition of plasticizers, *e.g.* citric acid, which promote polymer coalescence on the drug particles and hence promote the formation of spherical and smooth surfaced microspheres.
   The size of microspheres can be controlled by the rate of spraying, the feed rate of polymer drug solution, nozzle size, and the drying temperature. This method of microencapsulation is particularly less dependent on the solubility characteristics of the drug and polymer and is simple, reproducible, and easy to scale up.
7. **Phase Inversion Microencapsulation[22]:**
   The process involves addition of drug to a dilute solution of the polymer (usually 1—5%, w/v in methylene chloride). The mixture is poured into an unstirred bath of strong non-solvent (petroleum ether) in a solvent to non-solvent ratio of 1: 100, resulting in the spontaneous production of microspheres in the size range of 0.5—5.0m m can then be filtered, washed with petroleum ether and dried with air. This simple and fast process of microencapsulation involves relatively little loss of polymer and drug.

Characterization of microspheres:

A. Particle size, shape and surface morphology analysis [23-25]:
All the microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope. The average particle size was determined by using the Edmondson's equation D mean = εnd/εn, where n= number of microspheres observed and d= mean size range. The shape and surface morphology of the microspheres was studied by using a scanning electron microscope.

B. Entrapment efficiency[16,26] :
To determine the incorporation efficiency, 25 mg of propranolol loaded microspheres were washed with 10 ml of suitable solvent to remove the surface associated drug. The microspheres were then digested in 10 ml of suitable solvent for 12 h at room temperature (25±2 0) to release the entrapped drug. Drug content was determined spectrophotometrically.

C. Swelling index [27,28]:
Swelling index was determined by measuring the extent of swelling of microspheres in a particular solvent. To ensure the complete equilibrium, exactly weighed 100 mg of microspheres were allowed to swell in solvent for 34 h. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed by using microbalance. The Hydrogel microspheres then dried in an oven at 60° for 5 h until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula swelling index= (mass of swollen microspheres-mass of dry microspheres/mass of dried microspheres) ×100.

D. *In vitro* bioadhesion [29]:
Bio-adhesive properties of IN microspheres were evaluated using everted sac technique.

E. *In vitro* drug release [16,29] :
To carry out the *in vitro* drug release, accurately weighed drug-loaded microspheres were dispersed in dissolution medium in a beaker and maintained at 37±2° under continuous stirring at 100 rpm. At selected time intervals 5 ml samples were withdrawn through a hypodermic syringe fitted with a 0.4 mm Millipore filter and replaced with the same volume

of pre-warmed fresh dissolution medium to maintain a constant volume of the receptor compartment. The samples were analyzed spectrophotometrically.

F. *In vitro* diffusion studies [30]:

The *in vitro* diffusion study was performed using in *vitro* nasal diffusion cell.

G. Stability studies of microspheres [31, 32]:

All the batches of microspheres were tested for stability. The preparations were divided into 3 sets and were stored at 4° (refrigerator), room temperature and 40° (thermostatic oven). After 15, 30 and 60 days, drug content of all the formulations was determined by the method discussed previously in entrapment efficiency section.

Factors influencing drug entrapment efficiency of microspheres:

The drug entrapment efficiency of the microcapsule or microsphere will be affected by different parameters, Fig.1 illustrate the factors influencing drug entrapment efficiency.

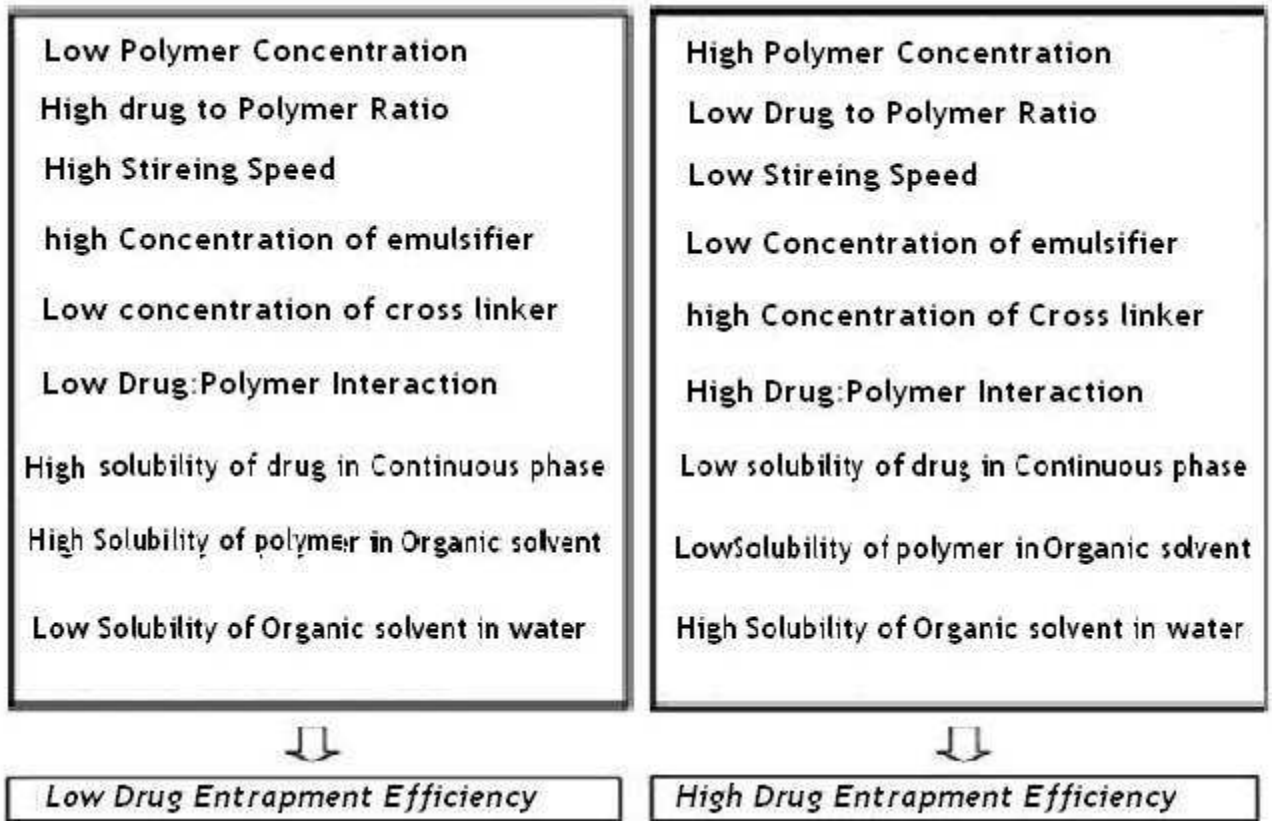

Figure1: Variables influencing the drug entrapment efficiency.

Concentration of the polymer in dispersed phase:

Encapsulation efficiency increases with increasing polymer concentration (Mehta et al., 1996; Rafati et al., 1997; Li et al., 1999)[6-8]. For example, the encapsulation efficiency increased from 53.1 to 70.9% when concentration of the polymer increased from 20.0 to 32.5% (Mehta et al., 1996)[6]. High viscosity and fast solidification of the dispersed phase contributed to reduce porosity of the microparticles as well (Schlicher et al., 1997)[9]. 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 (Rafati et al., 1997)[7]. Second, the high concentration increases viscosity of the solution and delays the drug diffusion within the polymer droplets (Bodmeier and McGinity, 1988)[10]. 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]. Decreasing the polymer concentration leads to reduction in loading efficiency due to maximum drug: polymer ratio and small size of microspheres which result in more loss of drug from surface during washing of microspheres [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.

Thakkar et al investigated the effect of polymer concentration on the encapsulation effeiciency 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 [33].

Drug to polymer Ratio:

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 [34]. 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 increase as the drug:polymer ratio decreased[35].

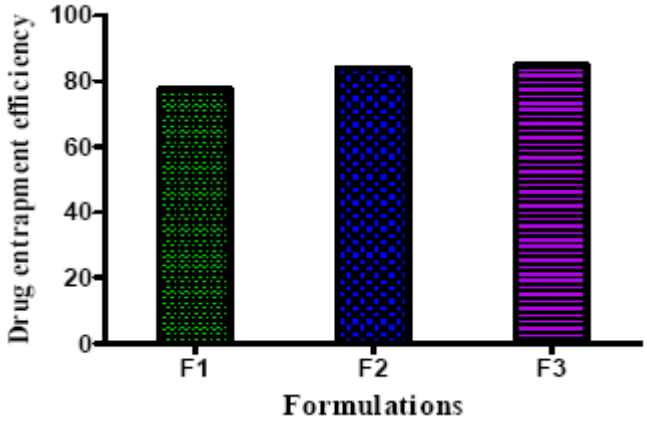

Figure 2: Drug entrapment efficiency of Trimetazidine hydrochloride Microspheres [37]

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 [36].

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 [37].

Solubility of polymer in the organic solvent:

Mehta et al., 1996[6], 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[6]. 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. Johansen et al., 1998 shown that the use of relatively hydrophilic PLGA which carried free carboxylic end groups resulted in significantly higher encapsulation efficiency compared to that of an end-capped polymer. A similar explanation as above applies to this observation: Hydrophilic PLGA is relatively less soluble in the solvent, methylene chloride, and precipitates more quickly than the end-capped one. High solidification rate might have increased the encapsulation efficiency [38]. On the other hand, the authors attribute the increase to the enhanced interaction between PLGA and the protein through hydrogen bonding and polar interactions7. Walter et al also observed increased encapsulation efficiency from using relatively hydrophilic PLGA in DNA microencapsulation [39]. The hydrophilicity of the polymer enhanced the stability of the primary emulsion, and it contributed to such an increase.

Solubility of organic solvent in water:

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 [10]. 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.

In order to explain the low encapsulation efficiency obtained with benzene, the authors mention that the benzene required a larger amount of water (non-solvent) than methylene chloride for precipitation of the polymer, and the drug was lost due to the delayed solidification. However,

given that benzene is a poorer solvent than methylene chloride for a PLA polymer, this argument does not agree with the widely spread idea that a poor solvent requires a smaller amount of non-solvent to precipitate a polymer. In fact, there could have been a better explanation if they had considered that the delayed solidification was due to the low solubility of benzene in water: As a poor solvent for a PLA polymer, benzene requires only a small amount of non-solvent for complete solidification of the polymer. However, since benzene can dissolve only a tiny fraction of water, it takes much longer to uptake water into the dispersed phase. That is, while solubility of a polymer in an organic solvent governs the quantity of a nonsolvent required in precipitating a polymer, solubility of the organic solvent in the non-solvent limits diffusion of the non-solvent into the polymer phase. Thus, when a co-solvent system is involved, both solubility of a polymer in a solvent and solubility of the solvent in a non-solvent participate in determining the solidification rate of the dispersed phase.

Park et al., 1998, lysozyme-loaded PLGA microparticles were prepared 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 (DP/ CP ratio):

Encapsulation efficiency and particle size increase as the volume of the continuous phase increases (Li et al., 1999, Mehta et al., 1996)[6,8]. 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 (Mehta et al., 1996) [6]. 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. A relevant observation is described in the literature (Sah, 1997) [42]. In this example, which utilized ethyl acetate as a solvent, the formation of microparticles was dependent on the volume of the continuous phase. When 8 mL of PLGA solution (o) was poured into 20 or 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. Microparticles generated from a low DP/CP ratio had a lower bulk density (0.561 g/cc at 1/50 vs. 0.357 g/cc at 1/ 300), which the authors interpret as an indication of higher porosity of the polymer matrix (Mehta et al., 1996)[6]. On the other hand, a different example shows that a higher DP/ CP ratio resulted in increased porosity, providing a large specific surface area (measured by the BET method) and the scanning electron microscope (SEM) pictures as evidence [40]. This apparent discrepancy can be explained by the fact that low bulk density [6] is not a true reflection of porosity but a result of large particle size. In fact, porosity increases with increasing DP/CP ratio, i.e., decreasing rate of the polymer precipitation.

Rate of solvent removal:

The method and rate of solvent removal influence the solidification rate of the dispersed phase as well as morphology of the resulting microparticles (Mehta et al., 1994)[43]. In the emulsion-solvent evaporation/extraction method, the solvent can be removed by (i) evaporation, in which the solvent is evaporated around its boiling point or (ii) extraction into the continuous phase. The rate of solvent removal can be controlled by the temperature ramp or the evaporation temperature in the former and by the volume of the dilution medium in the latter. PLGA microparticles containing salmon calcitonin (sCT) were prepared by emulsification, followed by different solvent removal processes [43, 44]. In the temperature dependent solvent removal process, the solvent (methylene chloride) was removed by increasing the temperature from 15 to 40oC at different rates. The microparticles that resulted from this process had a hollow core and a porous wall. The core size and wall thickness were dependent on the temperature ramp. A rapid rise in temperature resulted in a thin wall and a large hollow core, whereas a stepwise temperature rise (15 to 25, then to 40oC) resulted in a reduced core size. It is believed that the hollow core was due to the rapid expansion of methylene chloride entrapped within the solidified microparticles. Even though it is generally assumed that fast polymer solidification results in high encapsulation efficiency, this does not apply to the observation of Yang et al. [45]. Here, the encapsulation efficiency was not affected by the solvent evaporation temperature. It may be due to the different processing temperatures influenced not only the rate of polymer solidification but also the diffusivity of the protein and its solubility in water. While the high temperature facilitated solidification of the dispersed phase, it enhanced diffusion of the protein into the continuous phase, compromising the positive effect from the fast solidification.

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 [6]. For example, encapsulation efficiencies of more than 60% were achieved for salmon calcitonin (sCT) microparticles despite the high solubility of sCT in the continuous phase [40]. This is attributed to the strong affinity of sCT to hydrophobic polymers such as PLGA. On the other hand, such interactions between protein and polymer can limit protein release from the microparticles [41, 47, 48]. In certain cases, a co-encapsulated excipient can mediate the interaction between protein and polymer [38]. 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. Drug loss into the continuous phase occurs while the dispersed phase stays in a transitional, semi-solid state. 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. 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) [10].

Lee JH el prepared Water-soluble drugs were encapsulated within anionic acrylic resin (Eudragit® S100) microspheres by water in oil in oil (w/o/o) double emulsion solvent diffusion method. Dichloromethane and corn oil were chosen as primary and secondary oil phases, respectively. The presence of internal water phase was essential in forming stable emulsion droplets and it accelerated the hardening of microspheres. Results show that, the loading efficiency was >80% except for acetaminophen, due to its lower solubility in water and higher solubility in corn oil. As the volume of continuous phase increased the size of microspheres decreased [49].

Molecular weight of the polymer

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 distribution of the drug within microspheres was observed by a confocal laser scanning microscope. The encapsulation percentages of microspheres prepared from PLGA 15 000, 20 000 and 30 000 were 62.75, 27.52 and 16.63%, respectively [50]**.**

Effect of Different Stirring Rates on Drug Content:

The stirring rate of emulsion system is one of the frequently studied process parameters in microspheres technology. The effect of this parameter on biopharmaceutical properties of microspheres containing drug and matrix polymer was often observed.

Bozena et al was determined drug (Pipemidic acid) content for all selected size fractions of microspheres prepared at different stirring rates. Results are shown in Fig. 3.

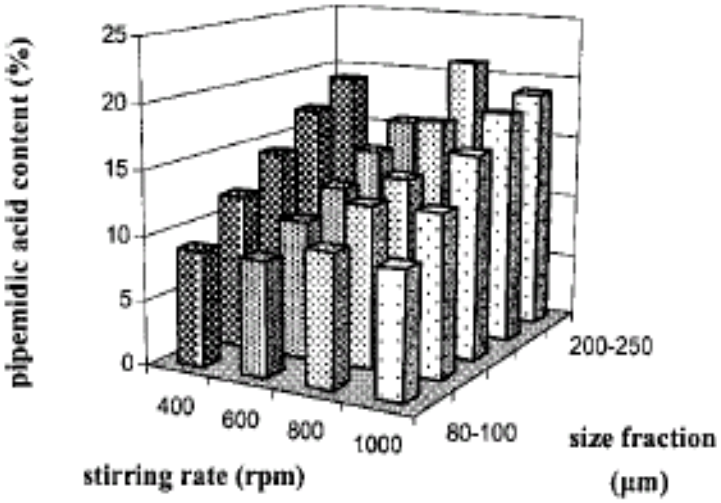

Figure 3: The Dependence of Drug(Pipemidic Acid) Content in Chitosan Microspheres on Their Fraction Particle Size and on the Stirring Rate[52]

The drug content (9—21%) increases with increasing particle size for each sample of microspheres prepared at different stirring rates. Furthermore, drug content determined for the biggest size fractions was higher than theoretical drug content in both series, although this effect was more expressed in the system without chitosan. Significance of the influence of particle size on drug content was also statistically confirmed (analysis of variance (ANOVA), p_0.001)[52].

Effect of concentration of emulsifier:

Thakkar et al investigated the effect of emulsifier on the encapsulation efficiency 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 [33].

There was a significant decrease in the entrapment efficiency with an increase in the concentration of span-85 from 2% wt/wt to 5% wt/wt. The decrease in the entrapment efficiency with an increase in the emulsifier concentration is because of dissolution of Celecoxib in the external phase of the emulsion at higher concentration of span-85. This decrease in the entrapment efficiency was more pronounced at a lower concentration of albumin [33].

Rawat et al studied the Influence of Selected Formulation Variables on the Preparation of Enzyme-entrapped Eudragit S100 Microspheres. Figure 4, 5 represent the response surface plot, which shows the effects of the X1 and X2 on the drug loading of microspheres. As can be seen through the response surface graphs, X2 is the most significant factor effecting drug content. Figure 3 expressed that, decreased drug loading as the concentration of Dichloromethane was increased [52].

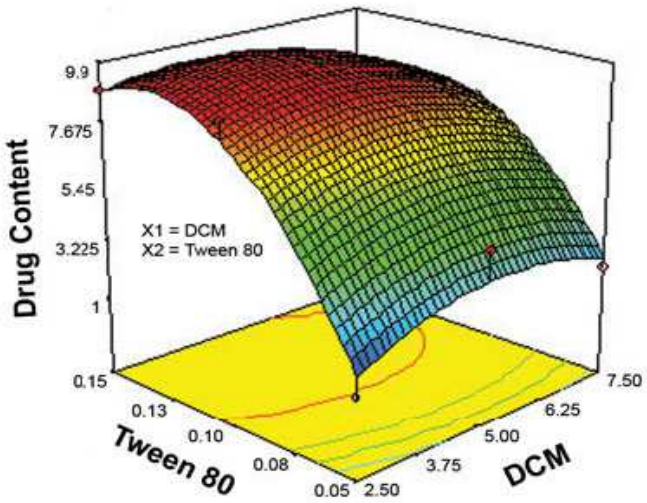

Figure 4: 3D surface curve for the effect of emulsifier (Tween 80) on the drug content of Microspheres [52]

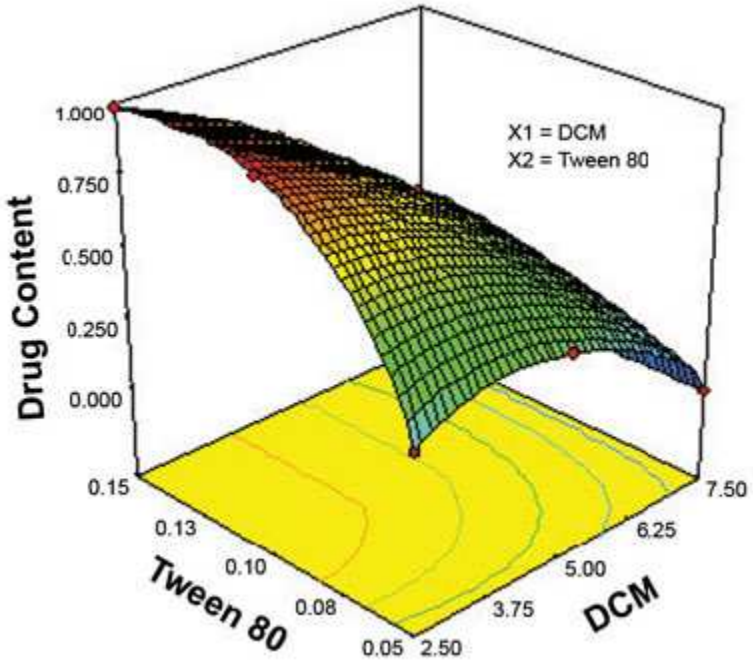

Figure 5: 3D surface curve for the desirable output in terms of maximum drug content and low level of DCM [52]

Effect Microcapsulation time:

Prajapati et al has investigated the effect of microcapsule formation time on loading efficiency of Gliclazide microspheres. Gliclazide microcapsules were prepared using sodium alginate and mucoadhesive polymer such as sodium carboxymethyl cellulose (sodium CMC), carbopol 934P or hydroxy propylmethyl cellulose (HPMC) by orifice-ionic gelation method.

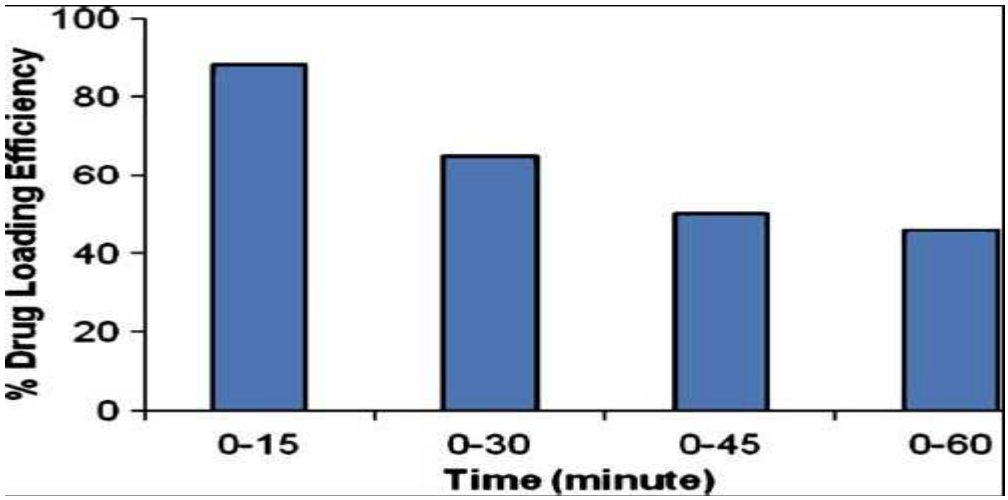

Figure 6: The effect of microcapsulation time on drug loading efficiency[53]

The loading efficiencies were found to be significantly affected by the time of microencapsulation. Loading efficiency increases as the time of microcapsule formation increases. The micro encapsulation efficiency for sodium alginate–sodium CMC was found higher compared to sodium alginate–HPMC and sodium alginate–carbopol 934P. The

microencapsulation efficiencies were found unaffected by the different ratios of polymer mixture [53].

Effect type of polymer:
Prajapati et al also invastiged the effect of type of polymer on loading efficiency of glipizide microspheres. The micro encapsulation efficiency for sodium alginate–sodium CMC was found higher compared to sodium alginate–HPMC and sodium alginate–carbopol 934P. The microencapsulation efficiencies were found unaffected by the different ratios of polymer mixture[53].

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[54].

Effect method of preparation:
The solvent evaporation method is popularly used for microsphere preparation because of its simplicity, reproducibility, and fast processing with minimum controllable process variables that can be easily implemented at the industrial level [55, 56]. But it is frequently used for water-insoluble drugs, as the 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 [14, 15].

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.

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