Available online at http://jddtonline.info

REVIEW ARTICLE

VARIABLES INFLUENCING DRUG RELEASE PATTERN OF MICROSPHERES: A TECHNICAL REVIEW

[1, 2]Ram C Dhakar*, [1]Sheo Dutta Maurya, [3]Rajeswar KK Arya, [4]Vikrant Saluja

[1]Dept. of Pharmacy, IEC Group of Institution, Greater Noida, INDIA
[2]Dept of Pharmaceutics, JJT University, Chudela, Jhunjhunu, INDIA
[3]Department of Pharmaceutical Sciences, Kumaon University, Nanital, INDIA
[4]Faculty of Pharmaceutical Sciences, PCTE Group of Insitutes, Ludhiana, INDIA
*****Corresponding Author's Email: dhakar_rc@yahoo.co.in*

Received 20 October 2011; Review Completed 27 Dec 2011; Accepted 27 Dec 2011, Available online 15 July 2012

ABSTRACT

Purpose of writing this review on microspheres was to compile the recent literature with special focus on the various variables which affect the drug release pattern 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 a carrier for drugs. For success of microspheres as drug delivery system, it's necessary they release the drug in controlled release manner for longer duration. This can be made possible by optimizing the formulation as well as process variables. Therefore before designing the microspheres formulation, it's necessary to understand the effect of various variables on the drug release pattern of these microspheres. The intent of the paper is providing the deep understanding of various variables those are useful during the development of microspheres system. This paper also summarize the various relevant aspects of microspheres

Key Words: Variables, Drug release pattern, Microspheres, Controlled release,

INTRODUCTION:

There are a number carriers – Microspheres[1], nanoparticles, liposomes and others for which optimized technologies are under development to a) enhance the performance of products that have already been delivered with some success via that route and b) modulates the release and absorption characteristics of the drugs particularly those drugs which have shorter biological half life. 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.

Of course, microspheres technology faces significant challenges. They include: accurately targeting the correct sites; increasing loading efficiency; the incorporation of optimum drug release kinetics and avoiding dose dumping. 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, 6-10], drug to polymer ratio, solubility of drug, dispersed phase to continuous phase ratio etc. These variables also affect the loading efficiency of the microspheres. Drug released from a microsphere can occurs in different way i.e. erosion, diffusion, swelling. Since the rate of drug release is controlled by the various factors, it is important to understand the physical and chemical properties of the releasing medium. Thus present review explains the effects of various variables on the characteristics of the release process. Additionally this also summarized the method of preparation and characterization of microspheres.

1. 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.

A. Emulsion cross-linking method[11]:

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

 ISSN: 2250-1177 CODEN (USA): JDDTAO

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.

B. Solvent Evaporation[12]:

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.

C. Hot Melt Microencapsulation[13]:

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.

D. Solvent Removal[14]:

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.

E. Hydrogel Microspheres[15]:

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.

F. Spray Drying[16]:

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.

G. Phase Inversion Microencapsulation[17]:

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.

2. CHARACTERIZATION OF MICROSPHERES:

A. Particle size, shape and surface morphology analysis [18-20]:

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 = ɛnd/ɛn, 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[11,21] :

 ISSN: 2250-1177 CODEN (USA): JDDTAO

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±20) to release the entrapped drug. Drug content was determined spectrophotometrically.

C. Swelling index [22,25]:

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[24]:

Bio-adhesive properties of microspheres were evaluated using everted sac technique.

E. *In vitro* drug release [11,24] :

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 50 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. Stability studies of microspheres[26, 27]:

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.

3. VARIABLES INFLUENCING DRUG release pattern OF MICROSPHERES:

There are following factors which directly/indirectly affect the drug release characteristics of the microspheres;

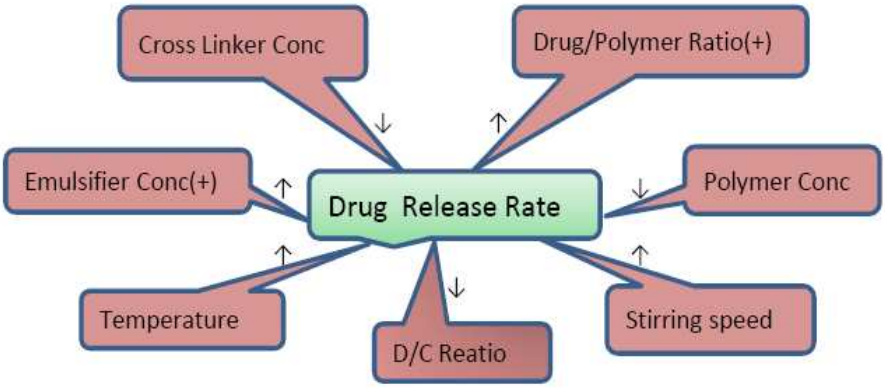

Figure 1: Variables affecting drug release from microspheres (↑→ Increase release rate, ↓→Decrease release rate)

A. 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.

Polymer concentration in aqueous phase indirectly affects the mucoadhesion time and drug release. As the polymer concentration in aqueous phase increases, size of microspheres is increased which results increase of mucoadhesion time and slower drug release from microspheres. [28]

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. [29]

The decrease in release rate with increasing content of the polymer can be explained by a decreased amount of drug present close to the surface and also by the fact that the amount of uncoated drug decreases with increase in polymer concentration [30]. When Eudragit® RL was used in combination with Eudragit RS, the drug released at a faster rate compared to Eudragit® RS alone. This is due to the fact that the amount of quaternary ammonium groups of Eudragit® RS is

 ISSN: 2250-1177 CODEN (USA): JDDTAO

lower than that of Eudragit RL, which renders Eudragit® RS is less Permeable [31].

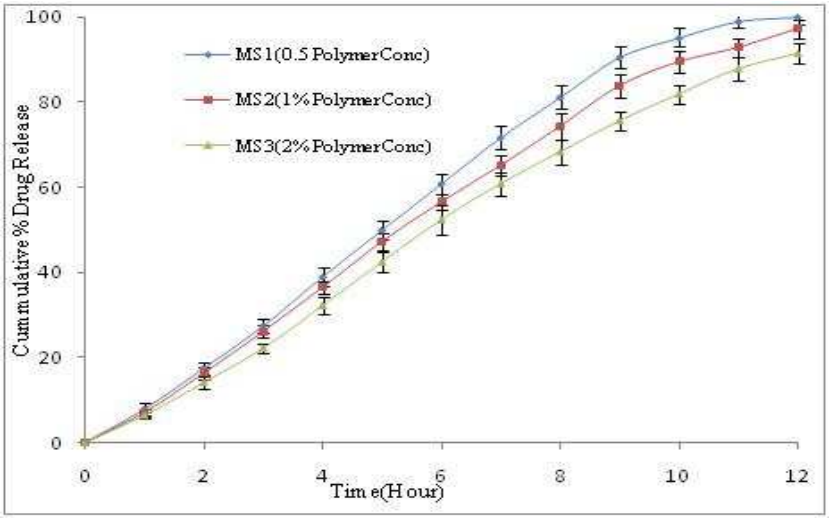

Figure 2: Shows effect of polymer concentration on Drug Release of microspheres[28]

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. [32]

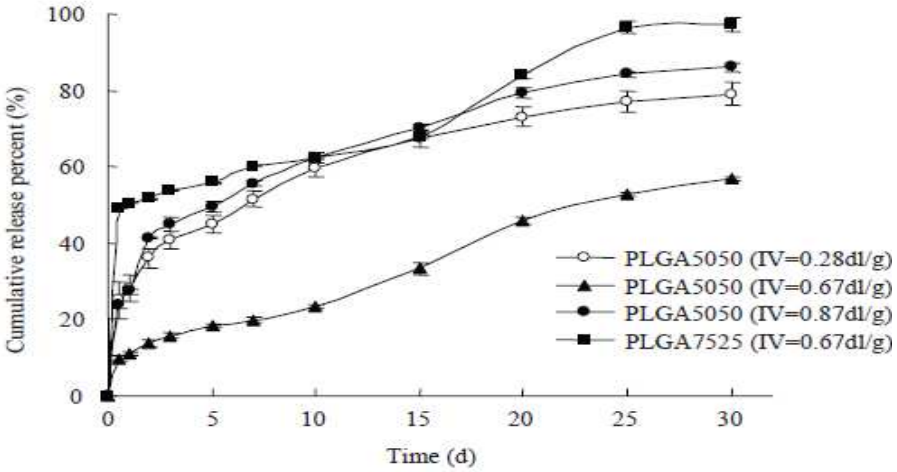

Figure 3: Effect of PLGA type on in vitro drug release [34]

Mazumder et al compare the dissolution profiles studies and found that drug release from cellulose acetate microspheres is faster than ethyl-cellulose microspheres. This could be due to high affinity to water for cellulose acetate than ethyl-cellulose. [33]

Dhakar et al studied that SCMC microspheres showed the faster drug release than drug release from HPMC microspheres due to rapid swelling property and high dissolution of SCMC in dissolution environment (0.1 N HCl) as compared to HPMC. Dissolution medium permeation in to the microspheres is facilitated due to high swelling action of the SCMC which leads to more medium for the transport of the drug is available.[1]

Yaju Ji et al investigate that typical drug release profiles of MEP421 PLGA microspheres exhibited significant "burst" release followed by slow drug release for over one month (Figure 3). Although, the microspheres with the high viscosity PLGA (IV = 0.87 dl/g) showed the lowest "burst" release (not more than 10% in the first 24 h), the drug release rate was also slow.

Even after 30 d of *in vitro* release, the cumulative drug release was less than 50% of the total drug loaded. Hence, polymer blends of PLGAs with various monomer ratios and viscosity were used as matrix materials for microspheres in further investigations in order to reduce the "burst release". By using blends of different viscosity PLGAs as matrix material for the microspheres, the burst release in the first 24 h could be limited to less than 20% of the total drug loaded, but the disadvantage was that the drug release rate was inevitably decreased at the same time (Figure 4). [34]

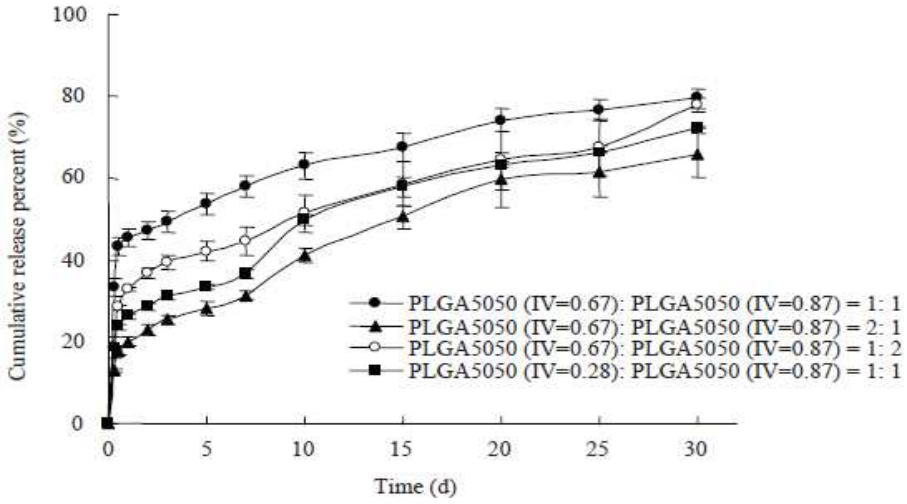

Figure 4: In vitro drug release of microspheres prepared using PLGA mixture [34]

Results from in vitro release tests show that clodronate release rate from microspheres seems to be affected mainly by copolymer MW. A significantly slower drug release rate is shown from microspheres of low MW copolymers compared to microspheres of high MW copolymers respectively. [35]

B. Drug: Polymer Ratio (DPR):

Effect of DPR on drug release from microspheres by Soni *et al* is shown that rate and extent of release is decrease with relative increase in the polymer concentration and this can be attributed to the increase in the density of the polymer matrix with increased polymer concentration. [36]

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 [37,38]. 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 [39].

At lower drug-polymer ratios, the mean particle size of the micropellets was less than that at higher drug-polymer ratios. Therefore, the drug release from micropellets prepared at lower drug- polymer ratios was faster than that of micropellets prepared at higher drug-polymer ratios because of the small size of the micropellets, which provided a large surface area for faster drug release. [40]

Dissolution profiles from study by Mazumder et al indicate that when drug to polymer ratio decreased from 1:1 to 1:3 a decreased in release rate was observed. It is considered that higher the drug to polymer ratio in the microspheres, result in increased in coat thickness surrounding the drug particles thereby increasing the distance traveled by the drug through coat [41-43].

C. Effect of Temperature:

Microspheres prepared at 60°C showed faster drug release than the microspheres prepared at 10°C. This can be attributed to the decrease in viscosity of the oily phase as the temperature increases, which in turn decrease the microspheres. [40]

D. Selection of solvent system for the dispersed phase

Selection of solvent system based on the volatility of solvent, solubility of polymer and type of method of preparation used for preparation of microspheres. Solvent should have high volatility and high polymer solubility.

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 [44]. Larger particle size indirectly slower the drug release from micoepheres.

E. 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 ($P < 0.05$) [45]. Similar phenomena were reported for the encapsulation of progesterone [45]. 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 [46]. Continuous phase containing a large amount of water resulted in faster polymer precipitation and therefore less porous spheres were formed [47].

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. [28]

Study from Mazumder shows that when the volume of external phase was increased from 50 ml to 100 ml, an increased in release rate significantly ($p < 0.05$ Student's t-test). This is due to higher migration of drug due to free movement of emulsion droplets, when the volume of external processing medium was increased. [33]

F. Effect of concentration of emulsifier:

Agrawal et al studied the effects of concentrations of emulsifier on the chracteristics of microspheres. The mean diameter of the microspheres at increasing concentrations of emulsifier (*i.e.*, 0.50, 0.75, 1.00, and 1.25%) decreased as 152.6, 147.1, 142.4, and 132.7 mm, respectively. The more emulsifier added, the less irregular were the microspheres, and the size of the microspheres was reduced. This appears to have resulted from a tightening of polymeric network, leading to microsphere shrinkage as the concentration of emulsifier is increased. Decrease in particle size results in faster drug release [29].

Mahboubian et al has prepared PLGA microspheres of triptoline. Results from study showed that higher amount of Span 20 (10%) led to a faster drug release rate by increasing the hydrophilic channels inside the hydrophobic PLGA matrix [48].

G. Effect concentration of cross linking agent:

Experimental cross-linking conditions (time and amount of the cross-linking agent) varyingly affected the Appearance of the microspheres surface, their mean particle size, drug loading and drug release of microspheres. The swelling ratio of microspheres increased dramatically when a smaller amount of cross-linking agent was used.

As shown in Figure 5, by increasing the concentration of glutaraldehyde-saturated toluene from 12 to 35% (V/V), the amount of drug release decreased from 30 to 10% in the first 30 minutes of the drug release experiment. The same pattern was observed when the duration of cross-linking was altered, *i.e.*, the longer the time of cross-linking the lower was the ratio of swelling. Results also shown that by decreasing the cross-linking time from 12 to 1 hour, the amount of drug release in the first 30 minutes was increased by 12 to 42% [49]. For batches cross-linked with 35% (*V/V*) glutaraldehyde-saturated toluene and 4 hours of cross-linking time period, t_{50} and t_{85} of drug release were 40 and 480 minutes, respectively. This type of release profile is of interest because the initial burst release can provide the initial penetration of lactic acid, and the sustained release phase supplies the skin with the drug over a prolonged period of time [50]. The initial burst drug release may be attributed to the release of drug molecules held loosely into or just beneath the surface of microspheres. Such a burst effect was reported previously for gelatin microspheres [51-52].

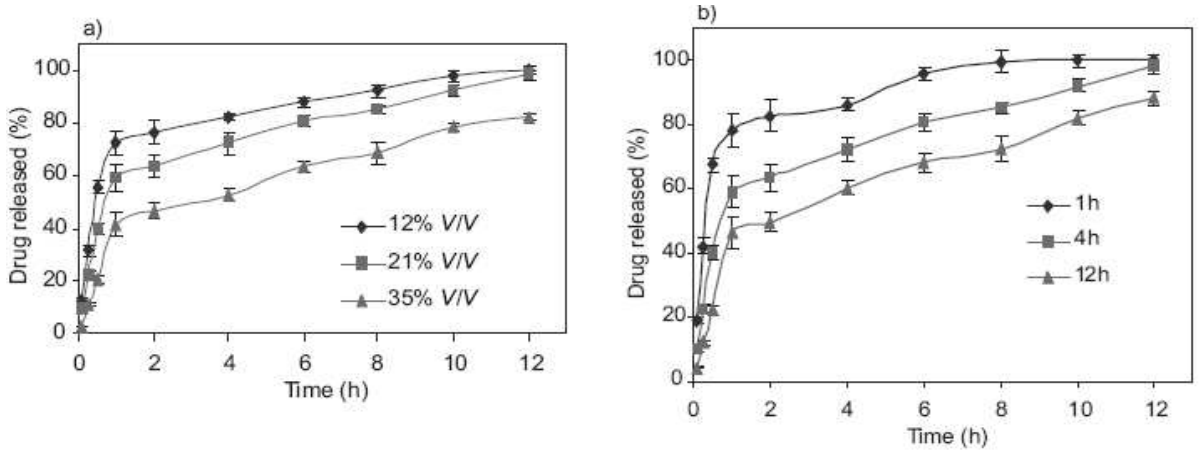

Figure 5: Effect of Concentration of Crosslink agent and cross linking time on drug release profile [49]

Tayade et al prepared the micropellets containing ibuprofen by cross-linking technique using gelatin polymer. The micropellets treated with formalin vapors for a longer period of time showed slower drug release as compared to micropellets treated for a shorter period of time. This result may be attributed to the higher degree of cross-linking of gelatin, when exposed to formalin vapors for a longer period of time, which retarded a drug release from the pellets[40].

H. Effect of stirring speed:

The drug release rate was increasing on increasing the stirring rate. Drug release was higher in the case of microspheres prepared at a higher stirring rate but at low stirring rate the release rate was slow. This can be attributing that smaller size microspheres have a larger surface area exposed to dissolution medium, giving rise to faster drug release [54]

Mazumder et al investigated that drug release was increased significantly ($p < 0.05$ Student's t-test) with increasing in stirring speed. When the stirring speed decreased from 1200 rpm to 600 rpm an initial burst release of around 38.45 % to 50.25 % occurred within 2 hours [33]. This can be attributed to the fact that the drug migration will be high for low stirrer speed and more amount of drug will remain in the microspheres surface but when stirring speed was increased drug migration will be less due to collision of emulsion droplets [54].

Dhakar et al studied the effect of stirring on the characteristics of metformin HCl microspheres. Results indicate that drug release rate is increased as increase in stirring speed. This is attributing the fact that increase in stirring speed leads to decrease in particle size which provide larger surface area for the dissolution [1].

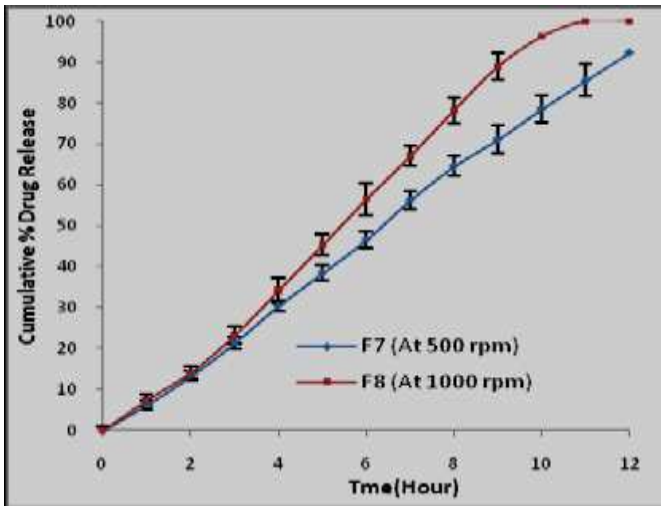

Figure 6: Effect of stirring speed on drug release of microspheres [1]

CONCLUSION:

For many drugs, microsphere technology offers an effective alternative to conventional oral and nasal delivery. The well designed microspheres technologies do away with various limitations i.e. poor surface morphology, low loading efficiency and unexpected drug release kinetics and open a completely new option for delivery of wide variety of therapeutics. Interestingly, literature survey revealed that the rates of Drug release significantly affect by polymer concentration, drug to polymer ratio, amount and type of cross linking agent, concentration of emulsifier, swelling index of polymer and many other variables. 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. This will only possible by understanding the effect of various variables which affect the drug entrapment efficiency of these microspheres. Progress to date suggests that microspheres technology can continue to expand and become an increasingly important drug delivery system for wide variety of therapeutics.

ACKNOWLEDGEMENT:

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

DECLARATION OF INTEREST: There are no declarations of interest.

REFERENCES:

1. Dhakar RC, Maurya SD, Aggarawal S, Kumar G, Tilak VK, Design and evaluation of SRM microspheres of metformin hydrochloride, Pharmacie Globale (IJCP), 2010, 1(6), 1-5.
2. Patel JK, Bodar MS, Amin AF, Patel MM, Formulation and optimization of mucoadhesive microspheres of metoclopramide, Indian J. Pharm. Sci., 2004, 66(3), 300-305.
3. Chowdary KPR, Srinivasa YR, Mucoadhesive microcapsules of glipizide: in-vitro and invivo evaluation, Ind. J. Pharm. Sci. 2003, 65(3), 279-284.
4. Chowdary KPR, Srinivasa L, Mucoadhesive drug delivery systems: A review of current status. Indian Drugs, 2000, 37(9), 400-406.
5. Benita S. Microencapsulation: Methods and Industrial applications. New York, NY: Marcel Dekkar; 1996.
6. Mehta RC, Thanoo BC, DeLuca PP, Peptide containing microspheres from low molecular weight and hydrophilic poly (D,L-lactide-co-glycolide). J. Controlled Release, 1996; 41: 249- 257.
7. Rafati H, Coombes AGA, Adler J, Holland J, Davis SS. Protein-loaded PLGA microparticles for oral administration: formulation, structural and release characteristics. J. Controlled Release, 1997; 43: 89-102.
8. Li X, Deng X, Yuan M, Xiong C, Huang Z, Zhang Y, Jia W, Investigation on process parameters involved in preparation of polylactide-poly(ethylene glycol) microspheres containing Leptospira Interrogans antigens. Int. J. Pharm., 1999; 178: 245-255.
9. Schlicher EJAM, Postma NS, Zuidema K, Talsma H, Hennink WE. Preparation and characterization of poly (D, L-lactic-co-glycolic acid) microspheres containing desferrioxamine. Int. J. Pharm., 1997; 153: 235-245.
10. Bodmeier R, McGinity JW, Solvent selection in the preparation of PLA microspheres prepared by the solvent evaporation method. Int. J. Pharm., 1988; 43: 179-186.
11. Sankar C, Mishra B. Development and in vitro evaluation of gelatin A microspheres of Ketorolac tromethamine for intranasal administration. Acta Pharm. 2003, 53:101-110.
12. Ogawa Y, Yamamoto M, Okada H, Yashiki T, Shimamoto T. A new technique to efficiently entrap leuprolide acetate into microparticles of polylactic acid or copoly(lactic/glycolic) acid., Chem. Pharm. Bull. 1998, 36: 1095-1103.
13. Mathiowitz E, Langer R. Polyanhydride microspheres as drug carriers. I. Hot-melt microencapsulation. J. Control. Rel, 1987. 5:13-22.
14. Carino PG, Jacob JS, Chen CJ, Santos CA, Hertzog BA, Mathiowitz E. "Bioadhesive Drug Delivery Systems—Fundamentals, Novel Approaches and Development," ed. by Mathiowitz E., Chickering D. E., Lehr C. M., Marcel Dekker, New York. 1999, pp459.
15. Lim F, Moss RD. Microencapsulation of living cells and tissues. J. Pharm. Sci. 1981, 70:351-354.
16. Bodmeier R, Chen H. Preparation of biodegradable poly lactide microparticles using a spray drying technique. J. Pharm. Pharmacol. 1988, 40, 754-757.
17. Chickering D, Jacob J. Preparation of biodegradable poly lactide microparticles using a spray drying technique.microspheres as oral drug delivery systems Biotechnol. Bioeng. Biotechnol. Bioeng. 1996, 52, 96-101.
18. Chen MJ, Wei W, Liu H, Bi D. Intranasal administration of melatonin starch microspheres. Int J Pharm. 2004, 272:37-43.

19. Martin A, Bustamante P, Chun AH. In: Physical pharmacy: Physical and chemical principles in the pharmaceutical sciences. 4 th ed. New Delhi; BI Waverly Pvt Ltd. 1996.
20. Chuang Y, Yen MK, Chiang CH. Formulation factors in preparing BTM-chitosan microspheres by spray drying method. Int J Pharm. 2000, 242:239-42.
21. Tabassi SA, Razavi N. Preparation and characterization of albumin microspheres encapsulated with propranolol hydrochloride. DARU. 2003, 11:137-41.
22. Patel JK, Patel RP, Amin AF, Patel MM. Formulation and evaluation of mucoadhesive glipizide microspheres. AAPS Pharm Sci Tech. 2005, 6:E49-55.
23. Soppimath KS, Aminbhavi TM. Water transport and drug release study from cross linked polyacrylamide grafted guar gum hydrogel microspheres for the controlled release application. Eur J Pharm Biopharm. 2002, 53:87-9.
24. Fandueanu G, Constantin M, Dalpiaz A et al. Preparation and characterization of starch/ cyclodextrin bioadhesive microspheres as platform for nasal administration of Gabexate Mesylate (Foy®) in allergic rhinitis treatment. Biomaterial. 2004, 25:159-70.
25. Pisal S, Shelke Mahadik VK, Kadam S. Effect of organogel components on in vitro nasal delivery of propranolol hydrochloride. AAPS Pharm Sci Tech. 2004, 5:63.
26. Rao YM, Devi KM, Rameshachary B. Stability study of Refampicin mucoadhesive nasal drops. Indian J Pharm Sci. 1999, 61 :(3), 66-70.
27. Kulkarni GT, Gosthamarajan K, Suresh B. Stability testing of pharmaceutical products: An overview. Indian J Pharm Edu. 2004, 38:194,202-20.
28. Dhakar RC, Maurya SD, VermA KK, Singh AK, Aggarwal S, Maurya G, Effect of formulation variables on characteristics of pioglitazone maleate microspheres, Int.J.Ph.Sci. 2(3); 2010.
29. Jain SK, Rai G, Saraf DK, Agrawal GP, the Preparation and Evaluation of Albendazole Microspheres for Colonic Delivery, Pharmaceutical Technology, December 2004, 66-70.
30. Alex R, Bodmeier R. Encapsulation of water-soluble drugs by a modified solvent evaporation method I. Effect of process and formulation variables on drug entrapment. Journal of Microencapsulation 2001; 7: 347-355.
31. Das SK, Das NG. Preparation and in vitro dissolution profile of dual polymer (Eudragit® RS 100 and RL 100) microparticles of diltiazem hydrochloride. Journal of Microencapsulation, 1998; 15: 445-452.
32. Lakshmana PS, Shirwaikar AA, Shirwaikar A, Kumar A, Formulation and evaluation of sustained release microspheres of rosin containing Aceclofenac, Ars Pharm, 2009, Vol.50 no. 2; 51-62.
33. Mazumder B, Dey S, Bhattacharya S, Sarkar S, Mohanta B, Studies on formulation and characterization of cellulose-based microspheres of chlorpheniramine, Arch Pharm Sci & Res Vol 1 No 1 66-74 July 2009
34. Ji Y, Dong W, Wang X et al, Studies on MEP421 PLGA microspheres: preparation and drug release, *Asian Journal of Pharmaceutical Sciences 2008, 3 (5): 211-216.*
35. Perugini P, Genta I, Conti B, Modena T, Pavanetto F. Long-term Release of Clodronate from Biodegradable Microspheres. *AAPS PharmSciTech*. 2001; 2(3): article 10.
36. Soni ML, Kumar M, Namdeo KP, Sodium alginate microspheres for extending drug release: formulation and in vitro evaluation, International Journal of Drug Delivery 2 (2010) 64-68.
37. Cardinal JR. Matrix systems. In: Langer RS, Wise DL, (editors). Medical applications of controlled release systems. Vol. 1. Philadelphia: CRC Press Inc., 1984; pp. 41-3.
38. Song SZ, Cardinal JR, Kim SW. Progestin permeability through polymer membrane, V: progestrone release from monolitic hydrogel devices. J Pharm Sci 1981; 70: 216-21.
39. Atyabi F, Mohammadi A, Dinarvand A, Preparation of Nimodipine Loaded Microspheres: Evaluation of Parameters, Iranian Journal of Pharmaceutical Sciences Summer 2005: 1(3): 143-152.
40. Tayade PT, Kale RD, Encapsulation of Water-Insoluble Drug by a Cross-linking Technique: Effect of Process and Formulation Variables on Encapsulation Efficiency, Particle Size, and In Vitro Dissolution Rate, *AAPS PharmSci*. 2004; 6 (1).
41. Jalsenjek I, Nicolaidou CF, Nixon JR. The in-vitro dissolution of phenobarbitone sodium from ethyl cellulose microspheres. J Pharm Pharmacol, 1976; 28, 912-914.
42. Mortada SM. Preparation of ethyl cellulose microcapsules using the complex emulsion method, Pharmazie, 1982; 37, 427-429.
43. Kim CK, Kim MJ, Oh KH. Preparation and evaluation of sustained release microspheres of terbutaline sulfate Int J Pharm, 1994; 106, 213-219.
44. Park TG, Lee HY, Nam YS. A new preparation method for protein loaded poly (D,L-lactic-co-glycolic acid) microspheres and protein release mechanism study. J. Controlled Release, 1998; 55: 181-191.
45. Mao Shirui, Yi Shi, Li L, Xu J, Schaper A et al, Effects of process and formulation parameters on characteristics and internal morphology of poly(D,L-lactide-co-glycolide) microspheres formed by the solvent evaporation method, European Journal of Pharmaceutics and Biopharmaceutics, 2008, 68, 214–223.
46. Yang Q, Owusu-Ababio G, Biodegradable progesterone microsphere delivery system for osteoporosis therapy, Drug Dev. Ind. Pharm. 2000, 26, 61–70.
47. WI L, Anderson KW, Mehta RC, Deluca PP, Kinetic and thermodynamic modeling of the formation of polymeric microspheres using solvent extraction/evaporation method, J. Control. , 1995, 37, 187–198.
48. Mahboubian*a A*, Kazem Hashemein*a S*, Moghadam SH, Atyabi*ad* F, Dinarvand R, Preparation and *in-vitro* Evaluation of Controlled Release PLGA Microparticles Containing Triptoreline, Iranian Journal of Pharmaceutical Research 2010, 9 (4):
49. Dinarvand R, Mahmoodi S, Farboud E, Salehi M, Atyabi F, Preparation of gelatin microspheres containing lactic acid –Effect of cross-linking on drug release, Acta pharm. 2005, 55, 57–67.
50. Jalon E, Blanco-Prieto MJ, Ygartua P, Santoyo S, PLGA microparticles: Possible vehicles for topical drug delivery, Int. J. Pharm. 200,1 226, 181–184.
51. Taylor KA, A simple colorimetric assay for muramic acid and lactic acid, Appl. Biochem. Biotech. 56 (1996) 49–58.
52. Ugwoke MI, Verbeke N, Kinget R, Microencapsulation of apomorphine HCl with gelatin, Int. J. Pharm. 148 (1997) 23–32.
53. Haznedar S, Dortun B. Preparation and in vitro evaluation of Eudragit microspheres containing acetazolamide Int.J. .Pharm.2004, 269:1, 131-140.
54. Mostaf S, Shahbazi M, Shafiee A. Formulation and in-vitro evaluation of. eudragitL100® microspheres of piroxicam Nature, 2008; 1544, 1-5.

 ISSN: 2250-1177 CODEN (USA): JDDTAO