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FORMULATION AND EVALUATION OF PREDNISOLONE LOADED MICROSPONGES FOR COLON DRUG DELIVERY: *IN-VITRO* AND PHARMACOKINETIC STUDY

Sonali *[1, 2], Rahul Pratap Singh [2] and Sunil Kumar Prajapati [3]

Institute of Pharmacy, Bundelkhand University [1, 2, 3], Jhansi- 284 128, Uttar Pradesh, India
Department of Pharmacology, Institute of Medical Sciences [1, 2], Banaras Hindu University, Varanasi- 221 005, Uttar Pradesh, India

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Correspondence to Author:

Sonali

Junior Research Fellow,

Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, 221 005, India

Email: sonalidoharey@gmail.com

ABSTRACT: The purpose of this study was to develop prednisolone-loaded microsponges for colon specific drug delivery. The microsponges formulations were prepared by quasi-emulsion solvent diffusion method employing eudragit RS 100 as a polymer. The compatibility of the drug with formulation components was established by Fourier Transform Infra-Red (FTIR) spectroscopy. Afterwards, microsponge formulations were prepared by gradually increasing the drug: polymer ratio. The surface morphology, particle size, production yield, and drug entrapment efficiency of microsponges were examined. Shape and surface morphology of the microsponges were examined using scanning electron microscopy. Particle size of prepared microsponges was observed in the range of 465 ± 12.5 to 82.2 ± 15 µm. The drug entrapment efficiency of the microsponges was found in the range of 53.38 ± 0.95 to 91.75 ± 1.60 %. The *in-vitro* dissolution studies of microsponges in the media with different pH (1.2, 7.4 and 6.8) showed that drug release in colon could be controlled by Eudragit RS 100. It was observed that the release kinetics on the basis of the highest r^2 values best fitted a zero-order kinetic model. Cumulative release for the microsponges over 8 h ranged from 48 - 87 %. Plasma drug concentration of drug was also studied for optimized formulation and C_{max}, T_{max}, and AUC (area under curve) was also observed.

INTRODUCTION: Over the last few years, extensive efforts have been focused on targeting a drug or drug delivery system in a particular region of the body for extended period of time, not only for local targeting of drugs but also for better control systemic drug delivery.

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Oral drug delivery targeting to the colon is valuable in treating diseases of the colon (ulcerative colitis, amoebiasis, Crohn's disease, carcinomas & infections) whereby high local concentration can be achieved while minimizing side effects that occur due to release of higher concentration of drug in the gastrointestinal tract or because of unnecessary systematic absorption [1, 2].

Several approaches are utilized for achieving colon targeting include use of pH-sensitive polymer, time-dependent formulation, bacterial degrading coating material, biodegradable polymer matrix and hydrogels and prodrugs [3, 6].

Various multiparticulate approaches include formulation in the form of pellets; granules have been developed to achieve targeted and sustained release of drugs in the colon. They provide many advantages over single-unit systems because of their small size [7].

The aim of present study was to develop prednisolone- loaded microsponges for colon targeting. Microsponges are one of the most useful devices to deliver material in an effective prolonged and safe manner. It was found that microsponges have unique dissolution and compression properties due to presence of sponge like texture.

Microsponge drug delivery system has many favourable characteristics which make it suitable as a drug delivery carrier such as enhance stability due to high degree of cross-linking, reduce side-effects due to targeted and modify drug-release, and also it protects the entrapped active ingredients from physical and environmental degradation. These are also capable to deliver pharmaceutical active ingredients efficiently at the minimum dose at targeted site which reduce severe systemic side-effects [8, 10].

Recent studies showed that microsponge offers a novel approach for colon targeting because there is no chemical modification of the active agent and this system is potentially applicable to a large group of chemically diverse agents for selective delivery to the colon, including laxatives, steroids, amino salicylic acids, etc. Additionally, *in vitro* studies have indicated that the microsponge system enhances the rate of dissolution of water-insoluble drugs.

By appropriately modifying the microsponge system, drugs like corticosteroids have been entrapped and release at rates several times higher than the conventional micronized versions of the drugs. Based on this fact, it is probable that such an effect may lead to an increase in effective drug levels within the colon with a consequent increase in absorption and blood levels, i.e., an increase in bioavailability.

Preliminary studies indicate that the microsponge particles bind to the rough surface of the intestinal mucosa. A combination of the enhanced rate of adsorption and dissolution should significantly enhance drug bioavailability [11, 13]. Microsponges can be prepared by two methods known as liquid-liquid suspension polymerisation method and quasi-emulsion solvent method. In this studies quasi-emulsion solvent diffusion method was used [14, 15].

Ulcerative colitis *(Colitis ulcerosa, UC)* is a form of inflammatory bowel disease (IBD). It is a disease of the intestine, specifically the large intestine or colon, which includes characteristic ulcers, or open sores, in the colon. . Ulcerative colitis is an intermittent disease, with periods of exacerbated symptoms, and periods that are relatively symptom-free [16, 17].

Prednisolone comes under the class of adrenocorticoid steroid (anti-inflammatory) and it is very slightly soluble in water. Prednisolone is the most commonly used steroids for ulcerative colitis treatments. Orally administered prednisolone is well absorbed from the proximal and distal intestine, relying on rapid hepatic metabolism to reduce systemic impact. Long term therapy of prednisolone produce severe side effect such as fluid retention on the face (moon face, Cushing's syndrome), acne, constipation, bloody or black tarry stools, muscle weakness, reddish-purple stretch marks on arms, rapid weight gain wounds that will not heal [18, 19].

Therefore, prednisolone-loaded microsponges are proposed to formulate for oral controlled released of drug that minimize proximal absorption, allow high drug concentration in the colon and reduced side-effect. Eudragit RS 100 is known as ammonio methacrylate copolymers and exhibits a very low permeability, enabling sustained release formulation and it has well established mucoadhesive characteristics [20, 21].

The objective of this work was to formulate, optimize and evaluate Prednisolone-loaded microsponges for colonic delivery.

Microsponges of Prednisolone were prepared with eudragit RS100 as a sustained release polymer, ethyl alcohol as a solvent, poly-vinyl alcohol as an emulsifying agent and tri-ethyl citrate as a plasticizer.

MATERIALS AND METHOD:

Materials: Prednisolone was purchased from Tulika International Ltd**.**, Madhya Pradesh, India. Eudragit RS 100 was gifted by Evonic Degussa India Pvt. Ltd., Mumbai. Tri-ethyl citrate was obtained from Camport, New Delhi, India. All other chemicals and solvent were of analytical reagent grade. Animal studies were performed according to protocols approved by Institutional Animal Ethics Committee (IAEC) Bundelkhand University, Jhansi, Uttar Pradesh, India.

Method: Prednisolone loaded microsponges were prepared by quasi- emulsion solvent diffusion method. The internal phase was contained eudragit RS-100 (50 mg) and triethylcitrate (TEC) with 1% w/v and then dissolved in 5 ml ethyl alcohol. TEC was used as plasticizer. This was, followed by addition of drug with gradual stirring (500 rpm). The internal phase was then poured into polyvinyl alcohol (PVA) solution in water, the external phase. After 3 h of continuous stirring the microsponges were formed due to removal of ethyl alcohol from the system. The microsponges were filtered and dried at 40 ^{0}C for 12 h. All the twelve formulations of Prednisolone-loaded microsponges of different drug: polymer ratio was prepared [22, 24] **(Table 1).**

TABLE 1: FORMULATION CHART OF PREDNISOLONE-LOADED MICROSPONGES

S No.	Formulation Code	Drug : Polymer ratio	Ethyl alcohol (ml)	Poly Vinyl Alcohol (%w/v)	Tri-ethyl citrate (ml)	Water (ml)
1	PDRS1	1:1	5	0.3	1	50
2	PDRS2	3:1	5	0.3	1	50
3	PDRS3	5:1	5	0.3	1	50
4	PDRS4	7:1	5	0.3	1	50
5	PDRS5	9:1	5	0.3	1	50
6	PDRS6	11:1	5	0.3	1	50
7	PDRS7	13:1	5	0.3	1	50
8	PDRS8	15:1	5	0.3	1	50
9	PDRS5 (a)	9:1	5	0.4	1	50
10	PDRS5 (b)	9:1	5	0.5	1	50
11	PDRS5 (c)	9:1	10	0.3	1	50
12	PDRS5 (d)	9:1	15	0.3	1	50

PDRS: Prednisolone-loaded Eudragit RS 100 microsponge

Note: Formulations 9 to 12 is prepared for variables study (effect of solvent and emulsifying agent on production yield, particle size and encapsulation efficiency. PDRS5 is optimized formulation which is used for variables study.

Drug-polymer identification studies (Compatibility studies): The drug and polymer compatibility was characterized by means of FTIR spectroscopy. The compatibility was checked by making physical mixture of drug and polymer (1:1) and then the FTIR analysis of the mixture was done. Interaction of drug and polymer does not exist if the peaks will not change in FTIR spectra of mixtures, and it will show similar peaks like pure drug and polymer FTIR **(Figure 2).**

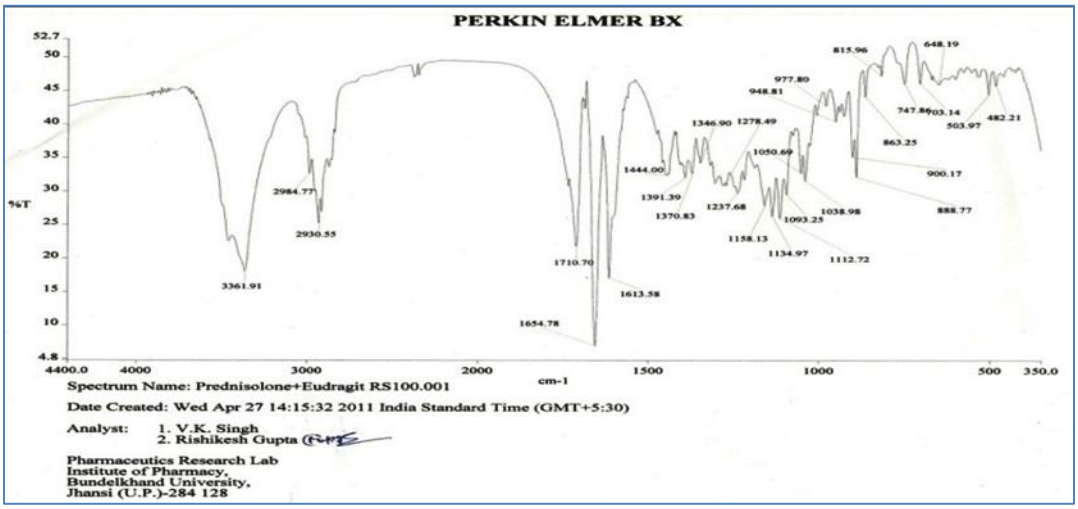
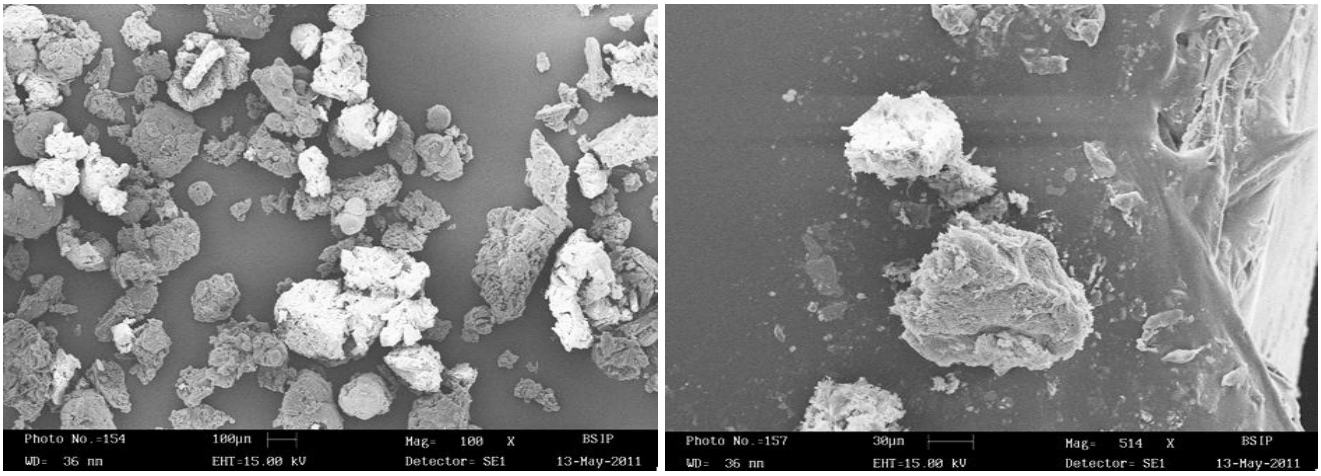


FIGURE 2: FTIR SPECTRUM OF PREDNISOLONE WITH EUDRAGIT RS 100

Microsponge Characterization:

Morphological examination (SEM): The surface morphology and structure were visualized by scanning electron microscopy (SEM). After gold coating samples were randomly scanned by using Zeiss DSM 982 Gemini, UK at Birbal Sahni Institute of Palaeobotany (BSIP) Lucknow, India, for particle size and surface morphology [15, 16] **(Figure 1a and 1b).**



1a **1b**

FIGURE 1a, 1b: SCANNING ELECTRON MICRO-GRAPHS OF MICROSPONGIC FORMULATION PDRS 5

Production yield: The percentage of production yield was calculated from the weight of dried microsponge (W_1) and the sum of initial dry weight of starting materials (W_2) as the following equation:

$$\% \text{ Production Yield} = W_1/W_2 \times 100$$

The determinations were done in triplicate [15, 16, 25] **(Table 2)**

Drug entrapment: In 100 ml volumetric flask, 25 mg of crushed microsponges were taken and dissolved with small quantity of ethanol and volume was made up to mark with phosphate buffer pH 6.8 and stirred for 12 hours.

After stirring, the solution was filtered through whatmann filter paper and from the filtrate, appropriate dilutions were made and absorbance was measured at 247 nm by using UV-spectrophotometer 1700 (Shimadzu).

The percentage drug entrapment was calculated by the following equation:

% Drug entrapment =

Calculated drug content / Theoretical drug content x 100

The determinations were done in triplicate [10, 13] **(Table 2).**

Particle size: The particle size and size distribution of the prepared microsponges was determined by using optical microscopy method. Approximately 200-300 microsponges were counted for particle size using a calibrated optical microscope (Olympus Pvt. Ltd., India) [10, 13] **(Table 2)**

TABLE 2: PRODUCTION YIELD, PARTICLE SIZE AND DRUG ENTRAPMENT OF PREPARED MICROSPONGES

Formulation	Production Yield (%) (mean ± S.D)	Particle size (µm) (mean ± S.D)	Drug entrapment (%) (mean ± S.D)
PDRS2	42.50 ± 1.60	465.68 ± 12.5	53.38 ± 0.95
PDRS3	60.00 ± 1.80	134.64 ± 13.5	68.04 ± 1.45
PDRS4	78.00 ± 0.70	132.10 ± 17.2	84.66 ± 0.60
PDRS5	92.00 ± 1.10	107.22 ± 9.6	90.22 ± 1.25
PDRS6	93.00 ± 1.35	104.58 ± 12.5	91.75 ± 1.60
PDRS7	80.28 ± 0.85	97.50 ± 10.0	75.56 ± 1.93
PDRS8	62.56 ± 1.90	82.20 ± 15.0	54.23 ± 1.70

n=3, S.D. =Standard Deviation

Optimisation of formulation parameters and process factors: The effect of drug: polymer ratio, concentration of emulsifying agent, volume of solvent and stirring speed was observed on particle size, production yield and drug entrapment efficiency.

***In-vitro* drug release study:** An *in-vitro* drug release study was carried out in United States Pharmacopoeia (USP) dissolution testing apparatus 2 (Paddle type) using treated dialysis membrane. The dissolution test was performed using 900 ml of simulated gastric fluid (pH 1.2). The content was rotated at 50 rpm at 37 ± 0.5 ^{0}C. Perfect sink conditions prevailed during the drug dissolution study period. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals.

The pH of the dissolution medium was maintained at 1.2 for 2 hours using 0.1 N HCl. Then dissolution medium was replaced with phosphate buffer and pH was maintained at 7.4 for next 2 hours. After 2 hours, the pH of dissolution medium was adjusted to 6.8 with 0.1 N NaOH and maintained up to next 4 hours. A 5 ml sample of the solution was withdrawn from the dissolution medium at regular interval by using a pipette fitted with a micro-filter and analysed drug release using a UV spectrophotometer (model 1700-E Shimadzu, Japan) at 247.5 nm. The volume of dissolution medium was maintained constant by replacing with equivalent volume of SGF after each withdrawal.

The concentration based on average calibration curves. All dissolution studies were performed in triplicate [25].

Application of different kinetic models for *in vitro* drug release: The result obtains from *in-vitro* dissolution data has been recognized as important parameters in the drug development. Under certain condition it has been used as substitute for the assessment of bioequivalence. Several theories and kinetic models (i.e. zero order, first order, matrix, Hixon-Crowell, Peppas and Korsemeyer etc.) describe drug dissolution from the immediate and modified release dosage form [26].

***In-vivo* study:** Plasma drug concentration study was performed on healthy female rabbits (2-3 Kg) in three groups (a) Group I-Control (b) Group II-Standard (c) Group III-Test. In first group no drug or formulation was given. Plain drug was administered to second group and optimized formulation PDRS5 was given to the third group. The rabbits were fasted overnight for 12 hours with free access to water. Water was given ad libitum during fasting and throughout the experiment. The rabbits were not anaesthetized during fasting and throughout the experiment.

5 mg of drug containing in enteric-coated capsules administered orally and they swallowed the formulation without any difficulty. The blood samples were collected from the marginal ear vein into centrifuged tubes just before dosing and at 1, 2, 3, 4, 5, 6, 7, and 8 hour after the drug administration. For the collection of blood samples, the rabbit ear vein was dilated by application swab. Blood samples were collected by means of a 1 ml syringe fitted with a 25 gauge needle. The needle, with the level in the upright position, was inserted at a 25^o to 30^o angle into the skin beside vein. The needle was lowered until it was almost flush with the skin aimed directly into the vein. Blood samples of 0.5 ml were collected in the specific time interval. The blood samples were collected in clean 2 ml centrifuge tubes without anticoagulant.

The blood was allowed to clot and the serum was separated by placing the tubes in a centrifuge 15 minutes at 2000 rpm. 100 µl serum samples were taken by micro pipette and diluted up to 2000 µl with phosphate buffer saline pH 7.4, the mixture was then firstly vortex then centrifuged at 2000 rpm, for 5 minute and supernatant was filtered through whatmann filter paper. The plasma drug concentration of prednisolone was analysed by UV spectrophotometric method. The mean and standard deviation (S.D.) was calculated by Graph Pad Instate 3.0. The statistical analysis was carried out employing analysis of variance (ANOVA) by using soft ware PRISM (Graph Pad) 5.0. Differences were considered statistically significant at $p < 0.05$ [27].

Stability studies of optimized formulation: The selected formulation of microsponges was stored in amber-coloured glass bottle at the different temperatures (4 ± 1^oC, 25 ± 1^oC and 50 ± 1^oC) for a period of 45 days and observed for any change in percentage residual drug content and *in-vitro* drug release. The samples were analyzed at the time interval of 7 days for one month. After every 7 days, percentage entrapment of the drug was determined in the formulations to know the amount of drug leaked out. The percent drug lost was calculated taking the initial entrapment of drug as 100 %.

RESULT AND DISCUSSION:

Preparation of Microsponges: The prednisolone-loaded microsponges were prepared by quasi-emulsion solvent diffusion method. This method was found to be very easy, reproducible, rapid method and avoid solvent toxicity [14]. In quasi-emulsion solvent diffusion method, the formation of microsponges could be described in the following processes: the formation of quasi-emulsion droplets, the diffusion of ethyl alcohol and the solidification of the droplets. The rapid diffusion of ethanol (good solvent for the polymer and drug) into the aqueous medium might reduce the solubility of the polymer in the droplets, since the polymer was insoluble in the water.

The instant mixing of the ethanol and water at the interface of the droplets induced precipitation of the polymer, thus forming the shell enclosing the ethanol and the dissolved drug. Counter diffusions of ethyl alcohol and water through the shell promoted further crystallisation of the drug in the droplets from the surface inwards. The dispersed droplets of polymer solution of drug were solidified in the aqueous phase via diffusion of solvent [15].

Microsponge formulations were prepared by gradually increasing the drug: polymer ratio. The effects of solvent, emulsifying agents and stirring speed on microsponge formulation were observed **(Table 1)**

Shape & surface morphology: The shape of microsponges prepared by quasi-emulsion solvent diffusion method was observed by SEM (Figure 1a & 1b). It was observed that the microsponges were spherical, and uniform with no drug crystals on surface. The shape of the microsponges affects the surface area and surface area per unit weight of spherical microsponges. The irregular shape of the particles may affect dissolution rate present in dissolution environment. The dispersion of the drug and polymer into the aqueous phase was found to be dependent on the agitation speed. The particle shape were irregular when the stirring speed was low because at low speed, lower energy produced and particles were stick together due to no formation of emulsion droplets.

As the speed was increased the shape of microsponges was found to be spherical and uniform. It was found that at optimized speed 500 rpm, spherical and free flowing microsponges are formed. But at very high speed the droplets breaks and irregular microsponges were formed [27, 28, 29]. The shape of microsponges was also affected by the amount of emulsifying agent. It was observed that on increasing the concentration of emulsifier, microsponges of large irregular shape was formed as seen by optical microscopy due to the increased viscosity. The concentration of 0.3 % of PVA was found to be optimized for producing spherical microsponges.

Particle size: The microsponges were found to be uniform in size**.** Particle size of prepared microsponges was observed in the range of 465 ± 12.5 to 82.2 ± 15 **(Table 2)** The sizes of microsponges affect the encapsulation efficiency and the release rate of drug. It was observed that as the ratio of drug to polymer was increased, the particle size decreased. This could probably be due to the fact that in high drug to polymer ratio, the amount of polymer available per microsponge was comparatively less. Probably in high drug-polymer ratios less polymer amounts surround the drug and reducing the thickness of polymer wall and microsponges with smaller size were obtained.

We have studied the effect of concentration of poly vinyl alcohol (PVA) on size of microsponges for optimized formulation (PDRS5). The selected concentration of PVA was 0.3 % but on taking 0.4 % of PVA particle size increases from 107.22 ± 22 µm to124 ± 12.5 µm. further on taking 0.5 % of PVA particle size increases to 135 ± 15 µm. The dispersion of the solution of the drug and polymer into droplets was affected by the concentration of polyvinyl alcohol in the external phase. When the concentration of PVA was increased, the size of microsponges was found to be increased due to the increased viscosity wherein larger emulsion droplets formed resulting in larger microsponges **(Table 3).**

TABLE 3: EFFECT OF EMULSIFYING AGENT ON PRODUCTION YIELD, PARTICLE SIZE, AND DRUG ENCAPSULATION EFFICIENCY

Formulation Code	PVA (%w/v) (mean ± S.D)	Production Yield (%) (mean ± S.D)	Particle Size (µm) (mean ± S.D)	Encapsulation efficiency (%) (mean ± S.D)
PDRS 5	0.3	92.00 ± 1.10	107.22 ± 9.6	90.22 ± 1.25
PDRS 5(a)	0.4	71.80 ± 1.20	124.00 ± 12.5	65.89 ± 1.25
PDRS 5(b)	0.5	54.80 ± 1.47	135.00 ± 15.0	47.10 ± 2.45

n = 3, S.D. = Standard Deviation

The effect of solvent volume, (ethyl alcohol) was also observed on the size of microsponges (Table 4). The result showed that on increasing the solvent volume particle size was decreased. The reason was investigated that the particle size was directly proportional to the apparent viscosity of dispersed phase. On increasing solvent volume from 5 ml to 15 ml, particle size was decreased from 107.22 ± 22 µm to 99.23 ± 10.4 µm due decrease in viscosity of solvent system. Same result was observed by [23].

Production yield: The production yield of the prepared microsponges of prednisolone was in the range of 42.5 ± 1.60 % to 93 ± 1.35 %. The loss of product was due to the formation of some agglomerates and polymer adherence to the container as a result of viscous nature of slurry. The formulation prepared with drug polymer ratio 7:1, 9:1, 11:1 showed good production yield, 78 ± 0.70, 92 ± 1.10, 93 ± 1.35 respectively. Further, on increasing drug polymer ratio (13:1, 15:1), production yield decreased. This could be due to less amount of polymer available for coating of high concentration of drug molecules **(Table 2 & Figure 3).**

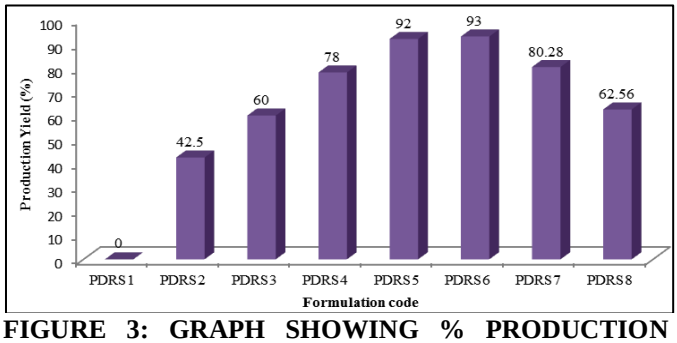


FIGURE 3: GRAPH SHOWING % PRODUCTION YIELD OF DIFFERENT FORMULATION

The effect of emulsifying agent (PVA) on the production yield of optimized formulation PDRS 5 (9:1) was studied. An increase in amount of emulsifying agent resulted in decreased production yield. On taking 0.4 % PVA, production yield decreased from 92.22 % to 71.8 %. Further on taking 0.5 % PVA production yield decreased. The reason of this may be that the polymer employed was non-ionic and molecule can associate away from oil-water interface at higher concentrations. Such alternative hydrophobic region can dissolve some portions of drug resulting in a reduction in production yield within microsponge formulation **(Table 3)**

It was also observed that when amount of ethyl alcohol (internal phase) was increased from 5 to 15 ml the production yield decreased (**Table 4)** This is due to lower concentration of drug in the higher volume of ethyl alcohol. The result was shown in table. In other microsponges was not formed on increasing the volume of internal phase from 5 to 10 ml [9].

Drug entrapment efficiency: Drug content in different formulations was estimated by UV spectrophotometric method. Basically, entrapment of the drug depends on the successful molecular association of the drug with the polymers. The drug entrapment efficiency of the microsponges was found in the range of 53.38 ± 0.95 to 91.75 ± 1.60 %. The best drug encapsulation efficiency was found for the formulation PDRS5 and PDRS6 with the drug polymer ratio of 9:1 and 11:1 respectively. On further increasing drug polymer ratio the encapsulation efficiency decreased. This could be due to high concentration of drug molecule in comparison to low concentration of polymer molecules which decreased the capability of polymer to coat the drug molecule and caused the reduction in encapsulation efficiency **(Table 2 & Figure 4)** When concentration of PVA increased from 0.3 % to 0.4 % and 0.5 %, the drug encapsulation efficiency was decreased. The reason was the same as in production yield **(Table 3)**

TABLE 4: EFFECT OF SOLVENT ON PRODUCTION YIELD AND PARTICLE SIZE OF OPTIMIZED FORMULATION PDRS5

Formulation Code	Solvent (ml)	Production Yield (%) (mean ± S.D)	Particle size (µm) (mean ± S.D)
PDRS5	5	92.00 ± 1.10	107.22 ± 9.6
PDRS5(c)	10	86.5 ± 2.67	104.2 ± 12.2
PDRS5(d)	15	77.4 ± 1.32	99.23 10.4

n=3, S.D. =Standard Deviation

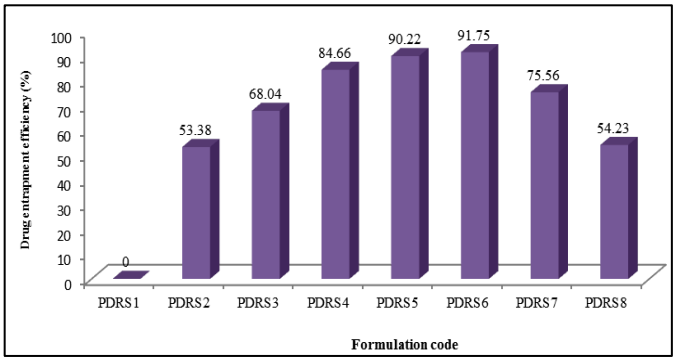


FIGURE 4: GRAPH SHOWING % DRUG ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATION

***In-vitro* drug release studies:** *In-vitro* drug release studies were performed in pH progression medium. Initially drug release was carried out in 0.1N HCl for 2 hours. After this drug release was carried out in phosphate buffer saline pH 7.4 for 2 hours followed by phosphate buffer pH 6.8 for next 4 hours **(Figure 5).**

The release profiles obtained for the formulation PDRS 3 to PDRS 8 are presented in **Figure 5.** It was observed that the drug release increases with increase in drug polymer ratio. This may be due to the fact the polymer concentration was kept constant for each formulation while the concentration of drug molecules was increasing which results in reduced thickness of polymer coat surrounding microparticles. The release showed initial burst effect due to non-encapsulated prednisolone in formulation. In the first 2 hours drug release was found to be 1.89 % to 2.71 % for PDRS 3, 2.73 % to 9.00 % for PDRS 4 and 3.7 % to 17.00 % for formulations.

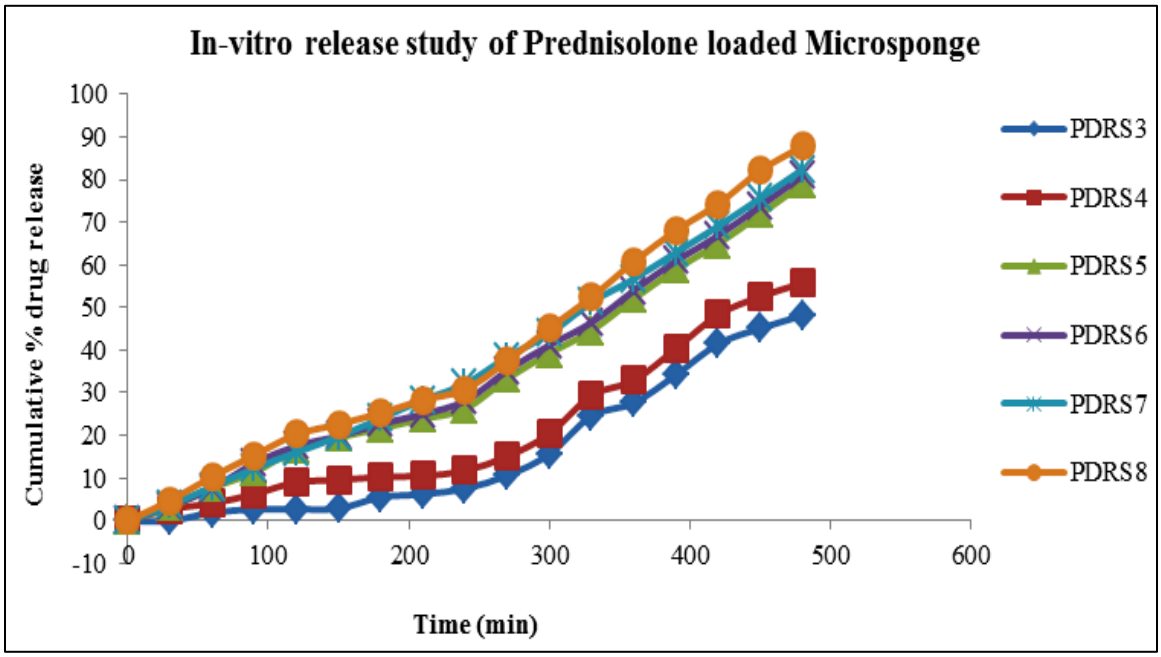


FIGURE 5: *IN-VITRO* DRUG RELEASE STUDY OF ALL FORMULATIONS

The burst effect could be due to two reasons: the first was the drug near or on the surface of the microsponges and the second was the well known porous nature of microsponges, the pores provided a channel for release of the drug. Because eudragit RS100 is a sustained release polymer, drug release was retarded by polymer coat in next 2 hours in phosphate buffer pH 7.4. Drug release was found to be 2.87% to 7.57 % for PDRS 3, 9.56 % to 11.76 % for PDRS 4 and 7 % to 30 % for formulation PDRS 5 to PDRS8. But in the next 4 hours, in phosphate buffer pH 6.8, maximum drug release was observed. The overall cumulative % drug release for PDRS 3, PDRS 4, PDRS5, PDRS 6, PDRS7, PDRS 8 were found to be 48.13 ± 0.32 %, 55.70 ± 0.12 %, 78.91 ± 0.19 %, 80.84 ± 0.67 %, 82.35 ± 0.65 %,87.81 ± 0.54 % respectively at the end of 8th hour.

The order of microsponges showing increasing cumulative % drug release was PDRS3 < PDRS 4 < PDRS 5 < PDRS 6 < PDRS 7 < PDRS 8. The cumulative amount released increased with an increase in concentration of active ingredient in the formula. But the release rate was higher in first 2 hours due to release of non-entrapped prednisolone while the release rate was observed slower for next 6 hours. This slower release rate is likely indication of the release of entrapped drug from micro-sponges.

The microsponges differ from regular microspheres with their highly porous surface. This characteristic gives property to release the drug at a faster rate through pores and this property is very helpful to get desired drug concentration at a targeted area or blood plasma.

According to previous research work, the microsponges having a more porous internal structure exhibited a faster drug release rate than that of rigid microsponges [14].

To study the release kinetics of prednisolone from the prepared microspheres, the goodness of fit method was applied and different kinetic equations were applied to interpret the release rate from the matrices. In the present study, the different nature of the curves obtained for zero-order, first order, and Higuchi model and as demonstrated by very close and highest r squared values suggests that the release from the formulation may follow any one of these models.

The results showed that the release kinetics on the basis of the highest r^2 values best fitted a zero-order kinetic model. The best fitted models were found to be Korsmeyer's for PDRS 3 and zero-order for PDRS 4, PDRS 5, PDRS 6, PDRS7 and PDRS 8 **(Figure 6 & 7)**

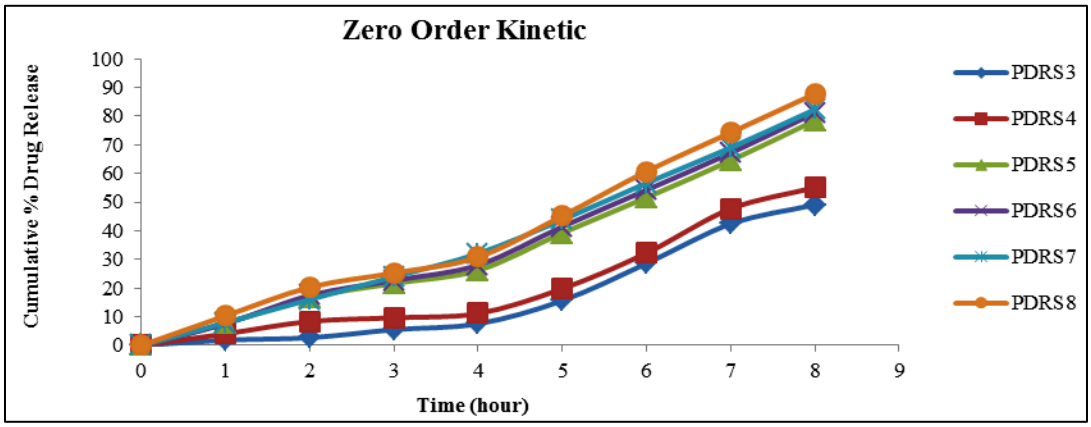


FIGURE 6: ZERO ORDER KINETIC TREATMENT OF OPTIMIZED FORMULATIONS

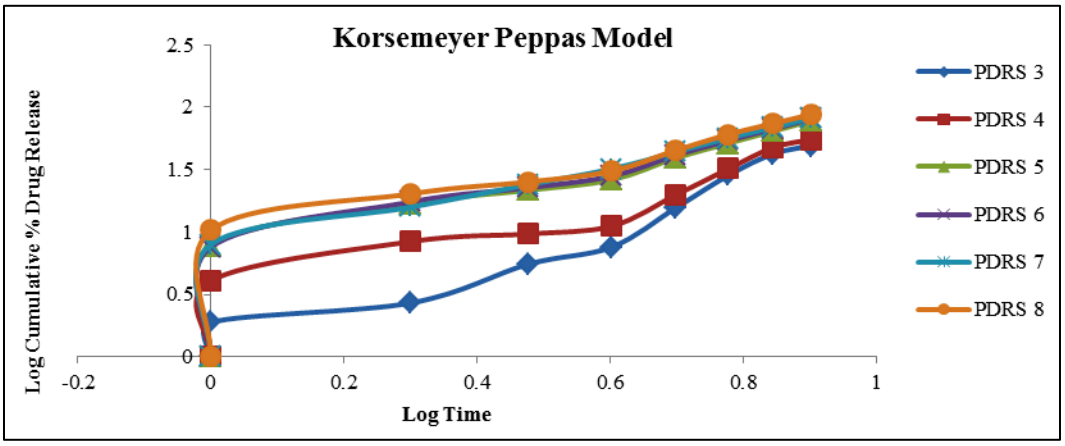


FIGURE 7: KORSEMEYER PEPPAS KINETIC TREATMENT FOR OPTIMIZED FORMULATION

***In-vivo* studies:** The *in-vivo* evaluation of prednisolone loaded microsponges was conducted in two groups of rabbit. Plasma drug concentration of Prednisolone was determined using UV spectrophotometric method. For plain drug, after oral administration the peak plasma concentration (C_{max}) of drug was found at the second hour and from the next hour plasma drug concentration declines rapidly.

This behavior of pure drug shows a typical peak and valley plasma concentration – time profile. But the formulation PDRS 5 which was given in enteric-coated capsule releases the drug at the third hour and peak plasma drug concentration reaches at the six hour. The formulation showed controlled kinetic profile. The observed $C_{max,}$ t_{max} and AUC (Area under curve) for the plain drug were 4.10 ± 0.2 µg/ml, 2.00 ± 0.15 hour and 9.49 µg h ml^{-1} respectively and $C_{max,}$ t_{max} and AUC for formulation PDRS5 were 3.65 ± 0.56 µg/ml, 6.00 ± 0.18 hour, and 13.16 µg h ml^{-1} respectively.

Thus, the results of the area under curve showed that the release of the drug from the formulation PDRS 5 showed controlled release pattern (**Figure 8**).

Stability Studies: The stability studies were applied on formulation PDRS5 because of their good encapsulation efficiency. The prednisolone microsponges were stored in glass bottles at 4 ± 1°C, 25 ± 1°C, and 50 ± 1°C, temperature for one month and evaluated at the interval of 10 days for any change in percentage drug content.

The percentage drug content varied from 99.88 ± 0.07 to 98.60 ± 0.19 at 5 ± 1°C, 99.76 ± 0.25 to 98.47 ± 0.07 at 25 ± 1°C and 99.66 ± 0.16 to 98.32 ± 0.21 at 40 ± 1°C. The decrease in drug content was found but it is in prescribed limits as per degradation phenomena. Thus, it was found that the optimized formulation PDRS5 was stable under storage conditions (**Figure 9**).

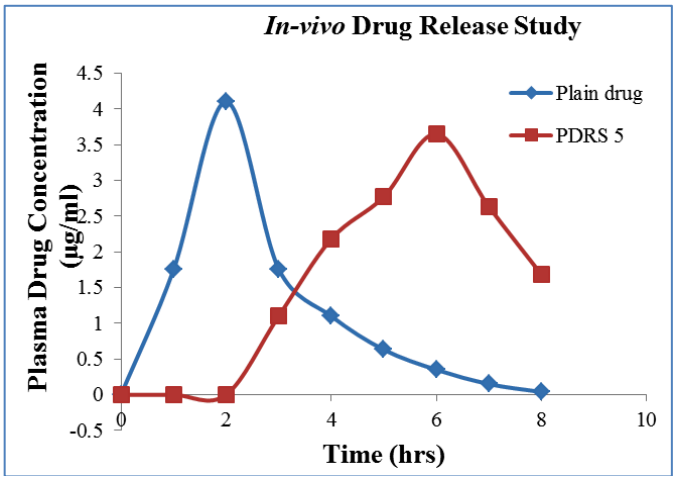


FIGURE 8: COMPARATIVE DRUG RELEASE STUDY OF PLAIN DRUG AND FORMULATION PDRS 5

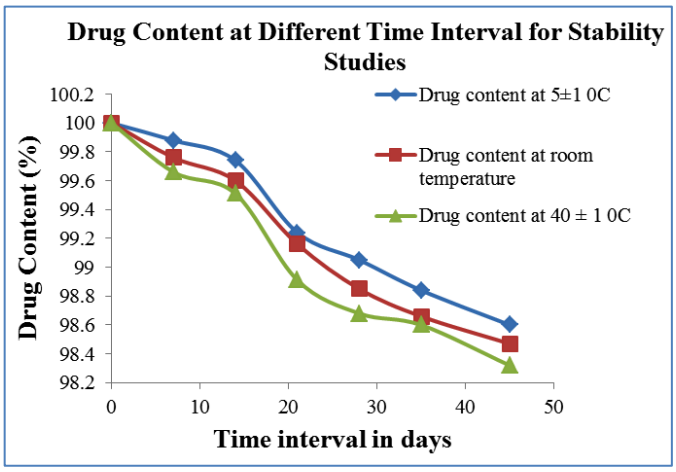


FIGURE 9: DRUG CONTENT OF FORMULATION PDRS5 AT DIFFERENT TIME INTERVAL FOR STABILITY STUDIES

CONCLUSIONS: Prednisolone-loaded eudragit RS 100 coated microsponges showed great advantage over conventional dosage form for colon targeting. It inhibits the release of drug in upper part of GIT and delivers it into colon and their allied area. In this study, microsponges were prepared by quasi-emulsion solvent diffusion method. This method was found to be easy, rapid, method and had an advantage of avoiding solvent toxicity.

In this research study, various studies have been included such as formulation development and evaluation, *in-vitro* and pharmacokinetic evaluation.

By infrared spectroscopic study, identity and purity of the drug was confirmed and it was cleared that there was no significant barriers to the development of the proposed formulation of the drug with the polymer and excipients. The obtained microsponges exhibited spherical shape and high porosity. The drug entrapment efficiency was found to reach up to 91 % in the formulations. This indicates that due to high porosity microsponges can entrap a large amount of drug thus increases bioavailability of drug. *In-vitro* dissolution studies were carried out by using USP XXIII dissolution test apparatus (paddle method). In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4, 6.8 were sequentially used referred to as sequential pH change method.

It was observed that no measurable drug release occurred up to 4 hours because of presence of time dependent polymer. Maximum release was found at pH 6.8 that is the pH of colon due to the presence of eudragit RS 100 which works as a sustained release polymer. Rate of drug release was much affected by drug: polymer ratio. Drug release was found maximum at higher drug: polymer ratio. Further microsponges are stable under storage condition.

The *in-vivo* studies performed in rabbits and draw a graph between the plasma drug concentration versus time which showed that the area under curve for the optimized formulation was more than that of plain drug, this indicate that the release of the drug from the formulation PDRS 5 showed controlled release. It has been concluded that obtained microsponges may work as a best dosage form for colon targeting due to good mucoadhesion property and more retention time in the colon. Microsponges are in porous nature so provide better controlled release and sufficient dose to the colon.

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REFERENCES:

1. Patel SB, Patel HJ, Seth AK: Microsponge drug delivery system: an overview of modified release chitosan, alginate and eudragit RS microparticles. J Chem Pharm Res 2010; 2(3): 704-721.
2. Srivastava R, Kumar D, Pathak K: Colonic luminal surface retention of meloxicam microsponges delivered by erosion based colon-targeted matrix tablet. Int J Pharm 2012; 427: 153– 162.
3. Vemula SK, Veerareddy PR: Different approaches to design and evaluation of colon specific drug delivery system. IJPT 2009; 1: 1-35.
4. Chauhan CS, Naruka PS, Rathore RS, Badadwal V: Formulation and evaluation of Prednisolone tablet for colon targeted drug delivery system. J Chem Pharm Res 2010; 2(4): 993-998.
5. Chandra D, Yadav IK, Jaiswal D, Ghosh N, Singh HP, Mishra A, Bhattacharya A, Bajpai M, Jain DA: Formulation and evaluation of satrinidazole microspheres for colon targeted drug Delivery. Journal of Pharmacy Research 2009; 2 (7): 1230-1233.
6. Arya P, Pathak K: Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: Optimization and pharmacokinetics. Int J Pharm 2014; 460: 1– 12.
7. Day NS, Majumdar and Rao MEB: Multiparticulate drug delivery system for controlled release. Tropical Journal of Pharmaceutical Research 2008; 7(3): 1067-1075.
8. Jangde R: Microsponges for colon targeted drug delivery system: an overview. Asian J. Pharm. Tech. 2011; 1(4): 87-93.
9. Mandava SS, Thavva V: Novel approach: microsponge drug delivery system. IJPSR, 2012; 3(4): 967-980.
10. Jain V, Singh R: Dicyclomine-loaded Eudragit®-based Microsponge with Potential for Colonic Delivery: Preparation and Characterization. Tropical Journal of Pharmaceutical Research, 2010; 9 (1): 67-72.
11. Orlu M, Araman A: Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. Int J Pharm 2006; 318: 103-117.
12. Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y: Control of prolonged drug release and compression properties of ibuprofen microsponges with acrylic polymer, eudragit RS by changing their intraparticulate density. Chem Pharm Bull 1992; 40: 196-201.
13. Gade R, Makineni A, Aparna.A, Keerthi. K, Murthy T.E.G.K, Rao CB, Nama S: Design and development of hydroxyzine hydrochloride controlled release tablets based on microsponge tecnology. Carib J SciTech 2013; 1: 172-184.
14. Perumal D: Microencapsulation of ibuprofen and Eudragit RS 100 by the emulsion solvent diffusion technique. Int J Pharm 2001; 218: 1-5.
15. TanselC, Baykara T: Preparation and *in-vitro* evaluation of modified release ketoprofen microsponges. Il Farmaco 2003; 58: 101- 106.
16. Patel R, Patel H, Baria A: Design: optimization and evaluation of pH responsive prednisolone sustained release tablet for ileo-colonic delivery. Webmed cent Pharm Sci 2010; 9: 1-5.
17. Ardizzone S: Drugs used in ulcerative colitis. Orphanet encyclopedia 2003; 1-3.
18. http://en.wikipedia.org
19. Chauhan CS, Naruka PS, Rathore RS, Badadwal V: Formulation and evaluation of Prednisolone tablet for colon targeted drug delivery system. J Chem Pharm Res 2010; 2(4): 993-998.
20. Mishra M K, Shikhri M, Sharma R, Goojar MP: Optimization, formulation development and characterization of Eudragit RS 100 loaded microsponges and subsequent colonic delivery. Int. J Drug Disc & Herb Res 2011; 1(1): 8-13.
21. Chandra D, Yadav IK, Jaiswal D, Ghosh N, Singh HP, Mishra A, Bhattacharya A, Bajpai M, Jain DA: Formulation and evaluation of satrinidazole microspheres for colon targeted drug Delivery. J Pharm Res 2009; 2 (7): 1230-1233.
22. Mohan kumar V, Veena N M, Manjula B P: Formulation and evaluation of microsponges for topical drug delivery of mupirocin. Int J Pharm Tech Res 2013; 5(3): 1434-1440.
23. Chandramouli Y, Firoz1 S, Rajalakshmi R, Vikram A, Yasmeen BR, Chakravarthi RN: Preparation and evaluation of microsponge loaded controlled release topical gel of acyclovir sodium. Int J Biopharm 2012; 3(2): 96-102.
24. Saboji JK, Manvi FV, Gadad AP, Patel BD: Formulation and evaluation of ketoconazole microsponge gel by quassi emulsion solvent diffusion. J Cell and Tissue Res 2011; 11(1): 2691-2696.
25. Huang B, Li GF, Luo JH, Duan L, Nobuki K, Akira Y: Permeability of rebamipide via rat intestinal membranes and its colon specific delivery using chitosan capsule as a carrier. World J Gastroenterol. 2008; 14(3): 4928-4937.
26. Patel JM, Brahmbhatt MR, Patel VB, Muley SV, Yeole PG: Colon targeted oral delivery of ornidazole using combination of pH and time dependent drug delivery system. Int J Pharm Res 2010; 2 (1), 28-35.
27. Jelvegiri M, Nokhodchi A: The microsponge delivery system of benzoyl peroxide: Preparation, characterization and release studies. Int J Pharm 2006; 308: 124-132.
28. Nokhodchi A, Jelvehgari M, Siahi MR: Factors affecting the morphology of benzoyl peroxide microsponges. Iran J Pharm Sci 2007; 38: 834–840.

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