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FORMULATION DESIGN & DEVELOPMENT OF ARTESUNATE NANOSPONGE

Patil Bhagyashree Subhash* and Dr. S. K. Mohite

Rajarambapu College of Pharmacy, Kasegaon. Dist-Sangli-415404. Maharashtra (India).

***Correspondence for Author: Patil Bhagyashree Subhash**

Rajarambapu College of Pharmacy, Kasegaon. Dist-Sangli-415404. Maharashtra (India).

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ABSTRACT

The objective of the present study was to produce controlled release Artesunate Nanosponges for topical and oral delivery. Nanosponges using three different polymers ethyl cellulose, Poly (methyl methacrylate) and Pluronic F-68 (poloxamer 188) were prepared successfully using PVA as surfactant by emulsion solvent evaporation method. The effects of different drug: polymer ratios, surfactant concentration, stirring speeds and time, sonication time on the physical characteristics of the nanosponges as well as the drug entrapment efficiency of the nanosponges were investigated. Particle size analysis and surface morphology of nanosponges were performed. The scanning electron microscopy of nanosponges showed that they were spherical in shape and spongy in nature. Effective targeted drug delivery systems have been a dream for long time. Site specific or targeted drug delivery is used to treat many diseases like cardiovascular disease, Osteo-diseases, hormonal deficiency diseases like Parkinson's disease, autoimmune diseases like arthritis, diabetes. The invention of nanosponges has become a significant step towards overcoming these problems. The sponge acts as a three-dimensional network. These small sponges can circulate around the body until they encounter the target site and stick on the surface and began to release the drug in a controlled and predictable manner which is more effective for a particular given dosage.

KEYWORDS: Artesunate Nanosponges, Poly (methyl methacrylate) and Pluronic F-68 (poloxamer 188).

INTRODUCTION

Nanosponges are porous polymeric delivery systems that are small spherical particles with large porous surface. These are used for the passive targeting of cosmetic agents to skin, there by achieving major benefits such as reduction of total dose, retention of dosage form on the skin and avoidance of systemic absorption. These nanosponges can be effectively incorporated onto topical systems for prolonged release and skin retention thus reducing the variability in drug absorption, toxicity and improving Bioavailibility. Nanosponges are a new class of materials and made of microscopic particles with few nanometers wide cavities, in which a large variety of substances can be encapsulated. These particles are capable of carrying both lipophilic and hydrophilic substances and of improving the solubility of poorly water soluble molecules .Nanosponges are tiny mesh-like structures that may revolution is the treatment of many diseasesand early trials suggest this technology is up to five times more effective at delivering drugs forbreast cancer than conventional methods. The nanosponges are encapsulating type of nanoparticles which encapsulates the drug molecules within its core. By the method of associating with drugs, the nanoparticles can be classified into encapsulating nanoparticles, complexing nanoparticles and conjugating nanoparticles. The first type is represented by nanosponges and nanocapsules. Nanosponges such as alginate nanosponge, which are sponge like nanoparticles containing many holes that carry the drug molecules. Nanocapsules such as poly(isobutyl-cyanoacrylate) (IBCA) are also encapsulating nanoparticles. They can entrap drug molecules in their aqueous core. The second category is complexing nanoparticle, which attracts the molecules by electrostatic charges. The third type is Conjugating nanoparticle, which links to drugs through covalent bonds. Nanosponges are obtained by suitable cross linking process and also by different organic and inorganic materials. Nano sponges can encapsulate various types of molecules by forming inclusion and non inclusion complexes.

Cross linking process

Highly cross linked cyclodextrins and highly cross linked polystyrene (natural derivative of starch)are used for the fabrication of nanosponges insoluble in water and commonest organic solvents, nontoxic, porous, stable above 300°c which may be used to encapsulate, carry and /or selectively release a great variety of substances.

Organic and inorganic materials

Some examples of Nanosponges formed by using organic andinorganic materials are Titanium or other metal oxide based nanosponges, silicon nanospongeparticles. Carbon coated metallic Nanosponges.

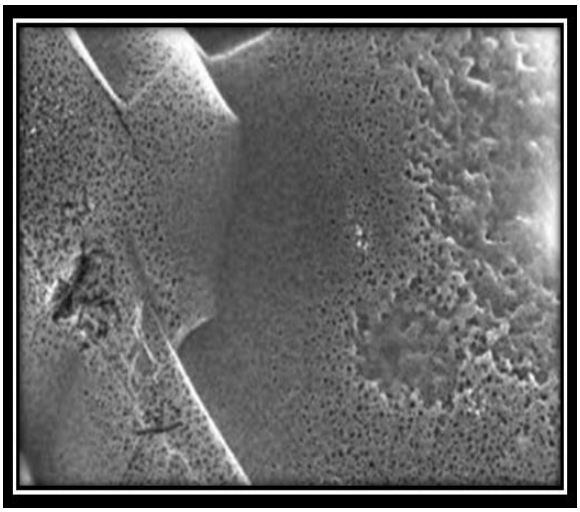
Interesting features of nanosponges

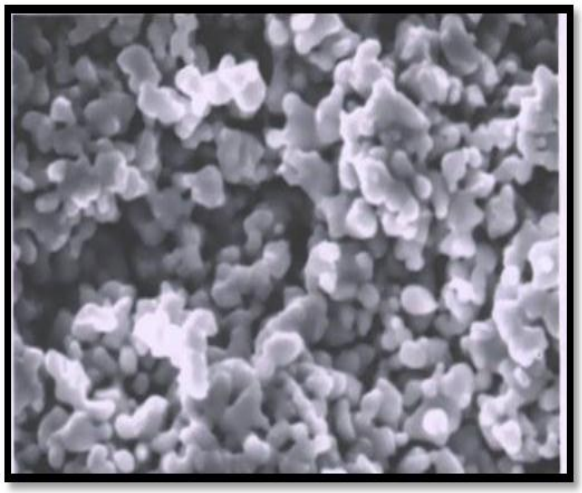
An important character of these sponges is their aqueous solubility; this allows The use of these systems effectively for drugs with poor solubility. The Nanosponges are capable of carrying both lipophilic and hydrophilic drugs. Nanosponges could be used to increase aqueous solubility of poorly water- soluble drugs, to remove pollutants from contaminated water, or as nano carriers for biomedical applications. Nanosponges have been used for removal of organic impurities in water. This technology offers entrapment of ingredients and reduced side effects, improved stability, increase delegance and enhanced formulation flexibility. Nanosponge and Nanosponge systems are non irritating and non-mutagenic, non-allergic and non-toxic. Extended release-continuous release up to 12h allows incorporation of immiscible liquid improves materia processing-liquid can be converted to powders. They can be formed in a sub micronsspherical particle. They can be obtained in a wide range of dimensions, from1micron to 10 microns. The cavities of the framew or k have a tunable polarity. Different functional groups can be linked to the structure due to sub micron dimensions of the particle. Nanosponge can disperse at molecular level, highly insoluble principles, stabilizing and protecting their structures, from chemicals, light, oxygen, etc. efficacy and shelf life of drugs can be prolonged if compared to the non-complexed form. By using Nanosponge as drug delivery system, higher therapeutic activities are observed being the concentration of the active molecule the same.

Advantages

This technology offers entrapment of ingredients and reduces side effects;

- Improved stability, increased elegance and enhanced formulation flexibility;
- Extended release with continuous action up to 12–24 hours;
- Incorporation of immiscible liquids is possible;
- Improved material processing since liquids may be converted to powders





Preparation methods of nanosponge

Preparation of nanosponges

Nanosponges prepared prepared mainly on the criteria of delivery system, polymer and nature of drug and solvents.

1) Nanosponges prepared from hyper-cross linked β-cyclodextrins

Prepared from β-cyclodextrins act as nanosporous materials performed their work as carriers for drug delivery. Due to this 3-d networks are formed which may be a roughly spherical structure about the size of a protein having channels and pores in the internal part. Reacting cyclodextrin with a cross linker such as di-isocianates, diaryl carbonates, carbonyl di-imidazoles etc. Sponges size is controlled according to porosity, surface charge density for the attachment to different molecules. Nanosponges are synthesized in neutral or acidic form dependon cross linker used. They consist of solid particales and converted in crystalline form. Capacity of nanosponges to encapsulate drug having different structures and solubility. They are used to increased aqueous solubility of poorly-water soluble drugs.

2) Emulsion solvent diffusion method

In this metod 2 phases are used in different proportion of organic and aqueous(ethyl cellulose and polyvinyl alcohol). The dispersed phase having ethyl cellulose and drug get dissolved in dichloromethane(20 ml) and a definite amount of polyvinyl alcohol added to 150 ml of aqueous continuous phase. Then, the mixture is stirred properly at 1000 rpm for 2hr. The required nanosponges were collected by the process of filtration and kept for drying in oven at 40°c for 24hr. Nanosponges which are dried were strored in dessicators and ensurity of removal of residual solvents is done.

3) Quasi-emulsion solvent diffusion

The nanosponges prepared using the polymer in different amounts. The inner phase is prepared using Eudragit RS 100 and added to a suitable solvent. Drug used provided with a solution and dissolved under ultrasonication at

35ºc. This inner phase added into external phase containing pva act as emulsifying agent. The mixture is stirred at 1000-2000 rpm for 3hr at room temperature and dried in an air-heated oven at 40ºc for 12hr.

Evaluation of nanosponges

1) Particle size determination

The size of particles are maintained during polymerization for the formation of free-following powders having fine aesthetic attributes. Particle size analysis of loaded and unloaded nanosponges performed by laser light diffractometry or malvern zeta sizer. Cumulative graph is maintained or ploted as particle size against time to study effect of particle size on drug release. Particles size greater then 30m impart gritty feeling and particles of sizes between 10 and 25 m preferred and used in final opical formulation.

2) Morphology and surface topography

For preparation of nanosponges in terms of morphology they are coated with gold-palladium under an atmosphere of orgon at room temperature and surface structure studied by scanning electron microscopy.

3) Determination of loading efficiency and production yield

The loading efficiency (%) of nanosponges calculated according to the equation The yield of nanosparticles can be determined by calculating initial weight of nanosponges.

3) Determination of true density

The repeated mean determination can be use to calculate true density of nanoparticles & benzoyl peroxide using ultra-pycnometer under helium gas.

METHODS

Calibration curve of Artesunate

Stock solution of Artesunate was prepared by dissolving 100 mg of accurately weighed amount of Artesunate 10 ml of distilled water and then the volume was adjusted to 100 ml with the same solution.

Procedure

The above stock solution of drug was subsequently diluted with distilled water to get 2 µg, 4 µg, 6 µg, 8 µg and 10 µg, of drug per ml. Then the absorbance of these dilute solutions was measured at a λ max of 284 nm by using double beam U.V. spectrophotometer against a blank of distilled water. Average of triplicate readings was taken and tabulated. Solubility studies. The solubility of Artesunate was determined in distilled water, different buffers, viz., pH 2.5, pH 7.5, pH 8.0 and pH 9.0. Triplicate readings were taken and average was calculated.

Solubility studies

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Preparation of nanosponge by solvent evaporation method

Five batches of nanosponges using different proportions of ethyl cellulose and polyvinyl alcohol were prepared by solvent evaporation method 11, 12. Disperse phase consisting of Artesunate and requisite quantity of ethyl cellulose dissolved in 10 ml solvent (dichloromethane or ethanol) was slowly added to a definite amount of PVA in 100ml of aqueous continuous phase, prepared by using microwave oven. The reaction mixture was stirred at 1000 rpm for three hours on a magnetic stirrer. The nanosponges formed were collected by filtration through whatman filter paper and dried in oven at 50^{o}C for 2 hours. The dried nanosponges were stored in vaccum desicator to ensure the removal of residual solvent.

EVALUATION OF NANOSPONGES

The Prepared nanosponges were evaluated for the following:

1. Characterization by scanning electron Microscopy
2. *In vitro* dissolution studies
3. Drug Entrapment efficiency

Drug Entrapment Efficiency

For the drug entrapment efficiency tests, the nanosponges of F1- F6 were performed. Before starting the chemical (spectrophotometric) analyses for the drug entrapment efficiency, the repeatability of measurements between different batches was ensured by repeated analyses. The 100mg of the nanosponge suspension was analyzed by dissolving the sample in 10ml of distilled water. After the drug was dissolved, 10 mL of clear layer of dissolved drug is taken. Thereafter, the amount of drug in the water phase was detected by a UV-spectrophotometric method at 284 nm (U.V Spectrophotometer, systronics). The test was repeated with another nanoparticulate sample.

***In vitro* Dissolution**

Apparatus - USP-II dissolution apparatus (Paddle) Medium - pH 6.8 buffer Temperature - 37^{o}C Time - 12 hours.

***In vitro* release studies**

In vitro release studies were performed in triplicate using USP Paddle method at 100 rpm and 37±0.2^{o}C in 900ml of phosphate buffer (pH 6.8). 100 mg of the formulated nanoparticles is used for each experiment. Samples were taken at appropriate time intervals for 5, 15 min interval in the first hour, half hour in next 3 hours, hourly sampling for up to 6 hours and finally for twelfth hour. The samples were measured spectrophotometrically at 284nm. Fresh dissolution medium was replenished each time when sample is withdrawn to compensate the volume.

Solubility studies

Solubility of Artesunate in distilled water, acidic and alkaline pH buffers was studied. It was found to be 100 mg/10ml in distilled water, 0.395 mg/ml in Ethanol, 100mg/10 ml in Dichloromethane but the solution was not clear.

Solubility by co-solvency

The solubility of drug in ethanol is increased by water as co-solvent, 100mg/ml.

Characterization

The nanosponges can be characterized for morphology by Scanning Electron Microscopy.

***In vitro* release studies**

In vitro release for the prepared nanosponges is done by using USP dissolution apparatus II. The samples are withdrawn at specific time intervals and analysed UVspectrometrically at 254nm. The release of drug was found to be more for F1 and F6 formulation, but F6 formulation drug release was found to be more sustained when compared with other formulation. The *in vitro* release of drug was found to be more in samples with less drying time. Though the other batches of formulation were found to release in sustained pattern, the F6 batch is considered as best sustained release of drug for 12hrs.

Drug Entrapment Efficiency

The drug entrapment efficiency tests, the nanosponges of F1- F6 were performed. Before starting the chemical (spectrophotometric) analyses for the drug entrapment efficiency, the repeatability of measurements between different batches was ensured by repeated analyses. The 100mg of the nanosponge suspension was analyzed by dissolving the sample in 10ml of distilled water. After the drug was dissolved, 10 mL of clear layer of dissolved drug is taken. Thereafter, the amount of drug in the water phase was detected by a UV-spectrophotometric method at 284 nm (U.V Spectrophotometer, systronics). The test was repeated with another nanoparticulate sample. The amount of the drug in the suspension was analyzed by centrifugation at 500rpm for 5 mins and by measuring the concentration of the drug in the clear supernatant layer by the UV-spectrophotometric method. The test was again repeated with another sample. The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting he amount of drug in the aqueous phase of the suspension from the total amount of the drug in the nanoparticle suspension. The entrapment efficiency (%) of drug was calculated by the following equation.

$$\% \text{ of Drug entrapment} = \frac{\text{Mass of drug in nanosponge}}{\text{Mass of drug used in formulation}} \times 100$$

Table no:-1 Calibration curve of Artesunate drug

Sr.no.	Concentration(µg)	Absorbance(289)
1	0	0.000
2	2	0.092
3	4	0.126
4	6	0.256
5	8	0.342
6	10	0.468
7	12	0.672

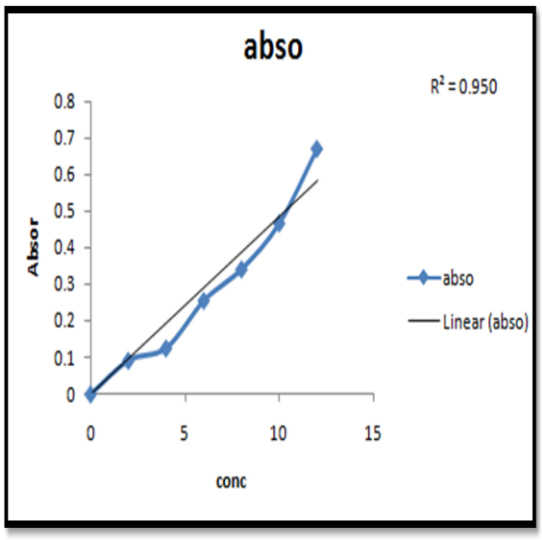


Table No. 2: Formulation aspects.

Sr.no	Materials	F1	F2	F3	F4	F5	F6
1	Artesunate	300mg	300mg	300mg	300mg	300mg	300mg
2	Dichloromethane	60ml	60ml	60ml	60ml	60ml	60ml
3	PVA	2gm	3gm	2mg	2mg	3gm	2gm
4	Drug : polymer	1:1	1:2	1:2	1;3	1:4	1:2
5	Ethylcellulose	300mg	600mg	600mg	900mg	1200mg	600mg
6	Water	20ml	-	-	-	-	20ml

Table No. 4: Drug Entrapment Efficiency.

Sr. No	Formulation	F1	F2	F3	F4	F5	F6
1	%Drug Entrapment Efficiency	89.68%	86.66%	90.12%	88.76%	92.44%	82.78%
2	Drying Time (hours)	1	2	1	1	2	1

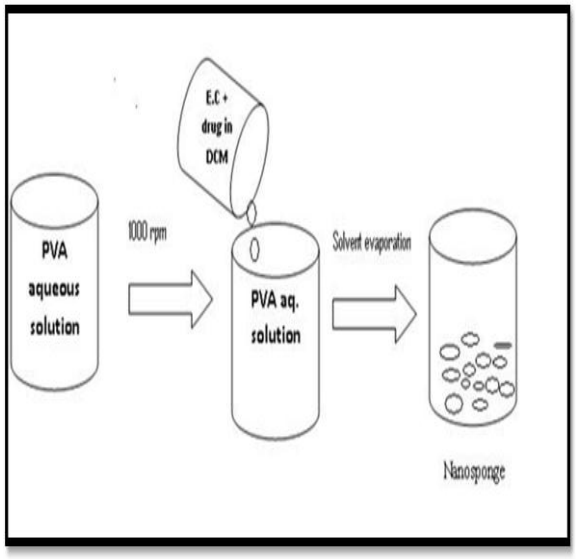
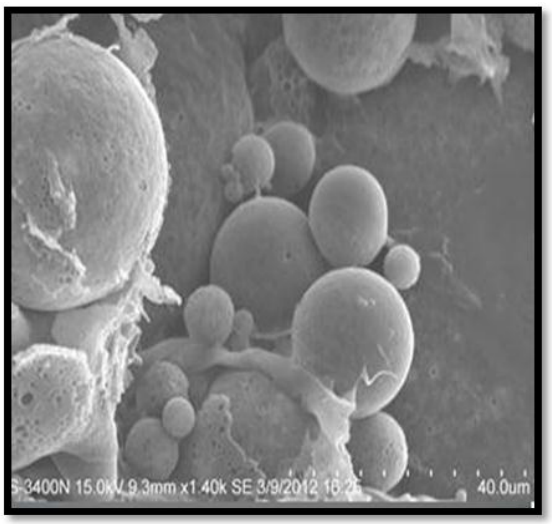


Figure No. 2: Preparation of Artesunate Nanosponge by Solvent evaporation method.

Figure No. 3: Formulated Artesunate Nanosponge (Batch-F5).



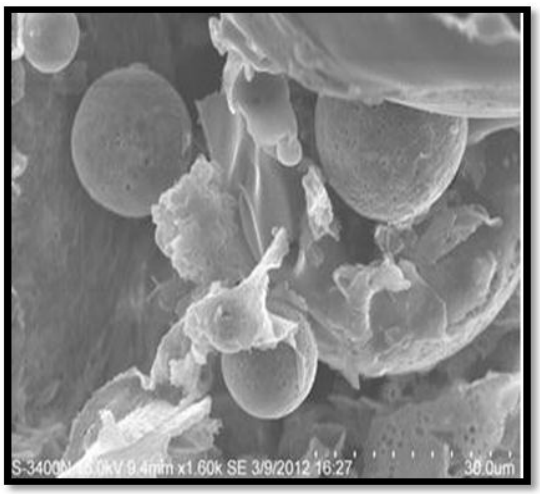


Figure No. 4: SEM Analysis photographs of Nanosponges.

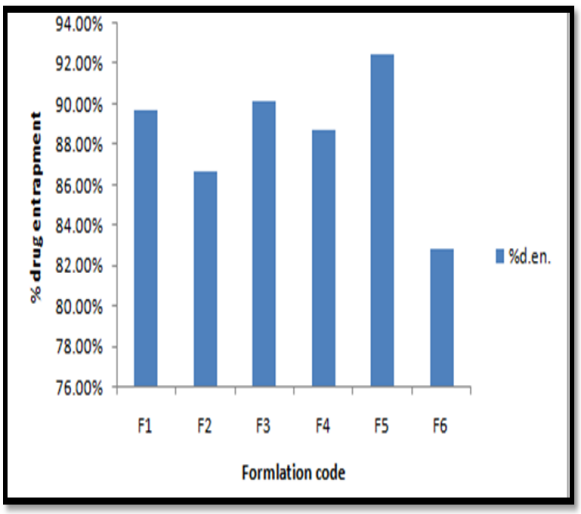


Figure no: % drug entrapment efficiency.

CONCLUSION

In the present study the formulated nanosponge loaded with Artesunate antibiotic resulted in sustained release. Among all the formulated batches starting from F1 through F6, the final batch (F5) is considered as the best entrapped (92.44%) nanosponge. The characterization by SEM finally concluded the appearance as a "Nanosponge".

BIBLIOGRAPHY

1. Eva Harth. Nanomedicine: Development of "Nanosponges" as superior sustain delivery systems of diverse biological cargos, Department of Chemistry, *Vanderbilt University, USA*, 2011.
2. Roberta C, Francesco T, Wander T. Cyclodextrin based nanosponges for drug delivery, *J inclphenom Macrocycl Chem*, 2006; 56: 209-213.
3. Khalid A A, Pradeep R V, Francesco T, Roberta C. Cyclodextrin dextrin - based nanosponges for delivery of Resveratol: *In vitro* characterization, stability, cytotoxicity and permeation study, *AAPS Pharm Sci Tech*, 2011; 12(1): 279-286.

4. Renuka S, Kamala P. Polymeric nanosponges as an alternative carrier for improved retention of Econazole nitrate onto the skin through topical hydrogel formulation, Department of Pharmaceutics, *Rajiv Academy for Pharmacy, Mathura, UP*, 2011; 16(4): 367-376.
5. Torne S J, Ansari K A, Vavia P R, Trotta F, Cavalli R. Enhanced oral Paclitaxe loaded Nanosponges, *Drug delivery*, 2010; 17(6): 419-425.
6. Isabelle A, Christine V, Helene C, Elias F, Patrick C. Sponge like alginate nanoparticles as a new potential system for the delivery of Antisense Oligonucleotides, *Anti sense and Nucleic acid Drug Development*, 1999; 9(3): 301-312.
7. David F. Nanosponge drug delivery system more effective than direct injection, *www.physorg.com*, 2011.
8. Renuka S, Roderick B W, Kamala P. Evaluation of the kinetics and mechanism of drug release from Econazole Nitrate Nanosponge loaded carbopol hydrogel, *Indian Journal of Pharmaceutical Education and Research*, 2011; 45(1): 25-31.
9. De Quan L, Min M. Nanosponges for water purification, *Clean products and processes*, 2000; 2: 112-116.
10. Selvamuthukumar S, Anandam S, Kannan Krishnamoorthy, Manavalan Rajappan. Nanosponges: A Novel class of delivery system - Review, *J Pharm Pharmaceut Sci*, 2012; 15(1): 111, 103-109.
11. Frances Separovic. Nanosponges for Targeted cancer treatment: The Promises of Nanotechnology, *Elizabeth Mudroch Theatre, University of Melbourne*, 2011.
12. http://chemicalland21.com/lifescience/phar/rabeprazole%20sodium.html.
13. Mallikarjuna Gouda M, Somashekar Shyale, Putta Rajesh Kumar and Shanta Kumar S M. Physico-chemical characterization, UV spectrophotometric analytical method development and validation studies of Rabeprazole Sodium, *J. Chem. Pharm. Res*, 2010; 2(3): 187-192.
14. Lala Rita. Current trends in β- Cyclodextrin based drug delivery systems, *IJRAP*, 2011; 2(5): 1520-1526.
15. Selvamuthukumar Subramanian, Anandam Singiredd, Kannan Krishnamoorthy and Manavalan Rajappan, Nanosponges:A Novel drug delivery system-Review, J Pharm Pharmaceut Sci (www.cspsCanada.org), 2012; 15(1): 103-111.
16. Gilardi G., Trota F., Cavalli R., Ferruti P., Ranucci E., Di Nardo G., Roggero C., Tumiatti V., Cyclodextrin nanosponges as carrier for biocatalysts, and in the delivery and release of enzymes, proteins, vaccines and antibodies, 2009; WO2009149883 A1.
17. Liang L., De-Pei L., Chih-Chuan L., Optimizing the delivery systems of chimeric RNA. DNA oligonucleotides beyond general oligonucleotide transfer. Eur. J. Biochem, 2002; 269: 5753–5758.
18. E. K. Patel, And R. J. Oswal, Nanosponge And Micro Sponges: A Novel Drug Delivery System, International Journal Of Research In Pharmacy And Chemistry, IJRPC, 2012; 2(2): ISSN: 2231-2781, 237-244.
19. Jenny A., Merima P., Alberto F., Francesco T., Roleof β- cyclodextrin nanosponges in polypropylene photooxidation. Carbohydrate Polymers, 2011; 86: 127–135.
20. Trotta F., Tumiatti V., Cavalli R., Rogero C., Mognetti B., Berta G., Cyclodextrin-based nanosponges as a vehicle for Anti-tumoral drugs, 2009; WO 2009/003656 A1.
21. Droz J.P., Chaladaj, A., Management of metastatic prostate cancer: the crucial role of geriatric assessment. BJU Int., 2008; 101(Suppl 2): 23–29.