Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 1027 - 1036

Research Article **Novel drug delivery system**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EFFECT OF PIPER NIGRUM ON IN-VITRO RELEASE OF ISONIAZID FROM ORAL MICROSPHERES

PRAHSANT L. PINGALE* AND RAVINDRA R.P.

SVKM'S NMIMS, School of Pharmacy and Technology Management, Shirpur- 425 405, Dist: Dhule, Maharashtra, India

ABSTRACT

The purpose of the study was to prepare and optimize a sustained release formulation of isoniazid microspheres and to study the effect of *Piper nigrum* (Black Pepper) on its *in-vitro* release. Microspheres containing isoniazid were prepared using double emulsification and complex coacervation methods. The critical formulation variables were concentration of polymer, drug - polymer ratio, cross-linking agent concentration and cross-linking time. The time to release 85% of the contents of the microspheres (t_{85}) was used as the measure for the release time of the drug. The microspheres were optimized on the basis of their particle size, percentage yield, entrapment efficiency, bioadhesion study and *in vitro* drug release. The *in-vitro* release of the optimized batches of isoniazid microspheres was enhanced to the extent of 100-107%, by coadministration of 10 or 15 mg of bioenhancer.

Key words: Tuberculosis, Bioenhancer, Piperine, Isoniazid, Microsphere

PRAHSANT L. PINGALE
SVKM'S NMIMS, School of Pharmacy and Technology Management, Shirpur- 425 405, Dist: Dhule, Maharashtra, India

INTRODUCTION

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* has afflicted the human race for centuries. Even though a vaccine and numerous effective antimycobacterial agents are available for its treatment, even in the 21st century, several million people still die from this disease each year[1]. The main problems associated with the anti-TB drug therapy include loss of efficacy through bacterial resistance, side effects, and low patient compliance[2,3]. In the development of ideal TB therapy, two points are considered to be important. First, the metabolism of *M. tuberculosis* is slow, resulting in a generation time that is measured in hours. This means that drug regimens should ideally have a low level of toxicity for long-term administration, and if possible, should be bactericidal so that elimination of the organism is rapid and is not totally dependent on the immune system. Second, the tubercle bacillus is a facultative intracellular parasite[4,5]. Therefore, drugs should also be able to penetrate host cells. Thus, an ideal method for treating tuberculosis would be one that not only is able to safely deliver drugs systemically for prolonged duration, but also would be able to target drugs to the intracellular environment in which the tubercle bacilli are found[6]. Isoniazid (INH) is a key component of the fixed dose combination (FDC) therapy used for first line therapy of TB as it can penetrate the host cells. However, the drug is characterized by a short half-life, ranging from 1 h to 4 h. Long-term continuous therapy with INH leads to hepatotoxicity and peripheral neuritis. It is thus important to design a formulation with controlled release of INH, especially in the small intestine[7] and with a much higher drug release (and consequently higher bioavailability). Bioenhancers are the substances which, when given along with other drugs in various treatments, enhance the bioavailability and bioefficacy of the drug without altering the drug properties. Hence they also reduce the toxicity and cost of the drug. A number of bioenhancers like *Piper nigrum, Piper longa, Carum carvi, Zingiber officinale* have been used with different class drugs like Anti-tubercular, Anti-leprosy, Anti-infective and Anti-cancer agents[8,9,10]. The exact mechanism of action of bioenhancers is unknown but they may act through various mechanisms, affecting mainly absorption, metabolism or on drug targets[11]. Microspheres are one of the multiparticulate drug delivery systems prepared to obtain prolonged drug delivery, to improve its bioavailability or stability and to target drug to specific sites. They may be defined as solid, approximately spherical particles ranging from 1 to 1000 μm, containing drug dispersed in either solution (or) microcrystalline form[12]. Sustained release microsphere delivery system releases the drug in the body in such a manner so as to keep the drug plasma concentration constant throughout the regimen.

The objectives of this study were to –

i. Develop sustained release microspheres of isoniazid using various methods;
ii. Study the effect of various concentrations of bioenhancer (extract of *Piper nigrum*) on *in-vitro* drug release from microspheres; and
iii. Evaluate the effect of various processing variables on characteristics of microspheres.

MATERIALS AND METHODS

Isoniazid was obtained as a gift sample from Lupin Pharmaceuticals Ltd., Aurangabad, Maharashtra. Black pepper (*Piper nigrum*) was purchased from local market and authenticated from Dept. of Botany, Dr. P. R. Ghogrey College of Science, Dhule, Maharashtra. Sodium alginate and Type B gelatin (bloom strength 220) were purchased from Loba Chemie, Mumbai. Sodium tripolyphosphate

(NaTPP) and Chitosan were purchased from Sigma Aldrich, Germany. All other chemicals / polymers were of analytical grade.

1. *Extraction and isolation of Piper nigrum used as bioenhancer*[13]

1.1. Macroscopy of Black pepper[14]: The entire fruit was almost globular in shape, with 4- 6.5 mm of diameter, brownish to black in color. The surface was found to be uneven. The seeds were almost brown or black in color, aromatic with a pungent taste. (Fig 1).

Figure 1
Piper nigrum plant with brown-black fruits and seeds

1.2 Phytochemical evaluation of Black pepper[15]: The phytochemical evaluation of fully mature, dried fruits of *Piper nigrum* Linn, family *Piperaceae* was carried out as per the provisions of Ayurvedic Pharmacopoeia for various parameters, the results of which are mentioned in Table 1.

Table 1
Phytochemical parameters of Black pepper

PARAMETER	RESULTS	LIMIT
Total ash	4.10 % w/w	Not more than 5.5% w/w
Water soluble ash	3.76 % w/w	Not more than 4.5 % w/w
Acid insoluble ash	0.57 % w/w	Not more than 1.0% w/w
Water soluble extractive	23.11 % w/w	Not less than 15% w/w
Methanol soluble extractive	10.50 % w/w	Not less than 8%w/w
Loss on drying	3.05 % w/w	Not more than 4 %w/w

1.3 Isolation of piperine from Black pepper[16]: Black pepper (20 g) was ground to a fine powder and extracted with 95% ethanol (150 ml) in a Soxhlet extractor for 2 hours. The solution was filtered and concentrated in vacuum on a water bath at 60°C. 10 ml of 10% alcoholic KOH solution was added to it and after a while, the clear liquid decanted from the insoluble residue. The alcoholic solution was left overnight, Yellow needle shaped crystals of piperine were obtained. The melting point of the crystals was found to be 124-126°C.

2. *Preparation of isoniazid microspheres*

Sustained release microspheres may be produced by several methods such as emulsion cross-linking method, multiple emulsion method, coacervation method, solvent evaporation method, spray-drying method etc. In this study, the double emulsification method[7] and complex coacervation method[17] were used to prepare the microspheres.

Method 1 - Double emulsification method

Isoniazid (75 mg) was dispersed in 3% aqueous solution of sodium alginate (10 ml). The aqueous phase was emulsified in light liquid paraffin (in the ratio 1:10) containing 1% (v/v) Span 80 using a mechanical stirrer (Remi Motors, India) at 1800 - 2200 rpm for 45 minutes. To it, 5 ml of 7.5% calcium chloride

dissolved in a mixture of methanol and isopropyl alcohol (1:2) was added slowly to the emulsion and stirred to assure efficient cross-linking. Microspheres were collected by filtration in vacuum, washed with isopropyl alcohol thrice and finally dried at room temperature. Variables like concentration of polymer, drug - polymer ratio, cross-linking agent concentration and cross-linking time were considered in the optimization of the formulation. Finally, varying concentration of bioenhancer, piperine was added to the optimized formulation to study its effect on bioavalability of drugs as shown in Table 2.

Table 2
Formulae of Isoniazid microspheres with variable polymer ratios and bioenhancer (double emulsification method)

Formulation code	INH (mg)	Na. Alginate (%)	$CaCl_2$ (%)	Cross linking time (min.)	Bioenhancer (mg)
MF1	75	3	7.5	45	--
MF2	75	3	7.5	45	5
MF3	**75**	**3**	**7.5**	**45**	**10**
MF4	75	3	7.5	45	15

Method 2 - Complex coacervation method
Chitosan and gelatin were dissolved in dilute acetic acid solution (1% v/v) together at concentrations of 3% w/v in ratio 1:05 and adjusted to a pH 5.0. Isoniazid (75 mg) was dissolved in the above polymeric mixture. The drug in polymeric mixture was emulsified in 100 ml of liquid paraffin (1:1 mixture of light and heavy liquid paraffin) at 40°C containing 1 ml Tween 80 (2% w/v). The emulsification was carried out for 15 min under mechanical stirrer (Remi Motors, India) at 1200 rpm. The w/o emulsion thus formed was cooled to 4°C to induce coagulation of gelatin. Then 50 ml Na-TPP (1.5% w/v) with pH 5 at 4°C was added drop wise. Stirring was continued for 30 min to obtain cross-linked microspheres. Microspheres were collected by centrifugation, washed with double distilled water thrice, then with acetone to remove water, and dried at room temperature under vacuum. The prepared microspheres were stored in desiccator for further studies. Concentration of polymer, polymer: copolymer ratio (chitosan: gelatin B), cross-linking time, rpm were considered as variables in optimization of the formulation. The composition of optimized formulation containing varying concentration of bioenhancer is shown in Table 3.

Table 3
Formulae of Isoniazid microspheres with variable polymer ratios and bioenhancer (Complex coacervation method)

Formulation code	INH (mg)	Chitosan: Gelatin (ratio)	NaTPP (%)	Cross linking time (min.)	Bioenhancer (mg)
CF1	75	1:0.5	1	30	--
CF2	75	1:0.5	1	30	5
CF3	75	1:0.5	1	30	10
CF4	**75**	**1:0.5**	**1**	**30**	**15**

3. Characterization of microspheres
Compatibility studies: Chemical interaction between the drug and the polymeric material, if any, during the preparation of the microspheres was studied by using Fourier Transform Infrared Spectroscopy (FTIR). Pure

drug INH, placebo microspheres, INH microspheres (2-5 mg) prepared with and without bioenhancer were weighed and mixed perfectly with potassium bromide (0.1 to 0.2 g) to form a uniform mixture. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR spectrum of the pellet were recorded using FTIR (Perkin Elmer, USA, Spectrum RX1 Model) taking air as the reference and compared with each other to identify drug-excipient interaction, if any.

Particle size analysis: Particle size of both plain drug microspheres as well as microspheres with bioenhancer was measured using Motic microscope at 40 X magnification. In all measurements, at least 100 particles in each of 3 different fields were examined.

Determination of percentage drug entrapment: The drug content of the microspheres was determined spectrophotometrically (λmax = 263 nm; Perkin Elmer, USA Lambda 25 model). Microspheres (10 mg) loaded with isoniazid were dissolved in 10 ml of isotonic phosphate buffer pH 6.8 under sonication for 20 min. The solutions were filtered through 0.22 µm Millipore filters and the amount of isoniazid was determined. Preliminary UV studies showed that the presence of dissolved polymers did not interfere with the absorbance of the drug at 263 nm.

The percent drug entrapment was calculated using following formula

$$\text{Percentage drug entrapment} = \frac{\text{Mass of drug present in microparticles}}{\text{Mass of drug used in the formulation}} \quad 100$$

Percentage yield: The yield of microspheres was determined by comparing the whole weight of microspheres obtained against the combined weight of the polymer, drug and bioenhancers used for formulation.

The percentage yield of the microsphere was determined using following formula

$$\text{Percentage yield} = \frac{\text{Wt. of microspheres obtained}}{\text{Total wt. of drug, polymer used for formulation}} \quad 100$$

Measurement of bioadhesion: In vitro bioadhesion was determined for microspheres (in triplicate) by falling liquid film method[18]. Microspheres (50 mg) were placed on albino rat small intestine (area 2cm^2) and kept for 20-30 minutes in a humidity temperature controlled cabinet (Thermolab, India), maintained at 75 (±5) % relative humidity and temperature of 25 (±2)^{0}C to allow hydration of the microspheres. This was followed by thorough washing of the mucosal lumen with isotonic phosphate buffer pH 6.8, and then dried at 70^{0}C in a hot air oven.

Percent bioadhesion was determined by the following formula

$$\text{Percentage bioadhesion} = \frac{\text{Wt. of adhered microspheres}}{\text{Wt. of applied microspheres}} \quad 100$$

In vitro drug release[7,19,20]: The release profiles of isoniazid from microspheres were studied in simulated gastric fluid (SGF pH 1.2) and simulated intestinal fluid (SIF pH 6.8). The drug-loaded microspheres (equivalent to 10 mg of isoniazid) filled in empty capsule shells were put

into the basket (50 rpm) and placed in 500 ml of the dissolution medium, thermostated at 37^0C. At scheduled time intervals the alliquots (2 ml) were withdrawn and replaced with fresh medium. The samples were diluted, filtered and the drug content determined spectrophotometrically at 263 nm. Statistical analysis: Statistical analysis of the results was carried out using Student's t-test. The in vitro release profile was compared with zero order, first order and Higuchi's matrix models.

RESULTS

Drug-polymer-bioenhancer interaction study: The FT-IR spectrum of INH showed a strong C=O stretch band (Amide I) around 1650 cm^{-1} and an Amide II due to N – H bend at 1620 cm^{-1}. These peaks were, however, completely masked in the FT-IR spectrum of the drug-loaded microspheres.

Particle size analysis: The microspheres had a smoother surface and were found to be discrete and spherical in shape (Fig. 2A). No change in the morphology was observed in drug-loaded microspheres (Fig. 2B). The mean particle size of the microspheres prepared by double emulsification method and complex coacervation method was found to be 109 - 125 µ and 118 - 128 µ respectively as shown in Table 6.

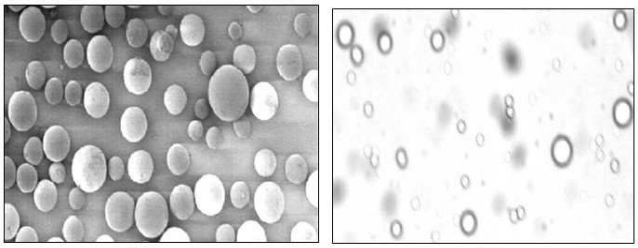


Figure 2.
Microspheres: A. by modified emulsification & B. by complex coacervation method

Determination of entrapment efficiency: The entrapment efficiency was found to be in the range of 43–82%. Loss of the drug in these methods may be due to loss in the hardening, washing and filtering processes. During optimization of microsphere formulation, it has been observed that the concentration of polymer, cross linker concentration and cross-linking time may affect the entrapment efficiency of the microspheres as shown in Table 4 (MC or CC = effect of croos linker concentration 'C') and Table 5 (MT or CT = effect of croos linking time 'T'). The entrapment efficiency of optimized formulations containing bioenhancer in various concentrations by both the methods is shown in Table 6.

Table 4
Effect of polymer and cross-linker concentration on microsphere entrapment

FORMULATION VARIABLES	FORMULATION					
	MODIFIED EMULSIFICATION			COMPLEX COACERVATION		
	MC1	**MC2**	MC3	CC1	**CC2**	CC3
Drug:polymer ratio	1:2	**1:4**	1:4	1:2	**1:4**	1:4
Cross linker concentration (%)	5.0	**7.5**	10.0	1.0	**1.5**	2.0
Entrapment efficiency (%)	64.20	**74.10**	69.70	61.70	**72.30**	67.40

Table 5
Effect of cross-linking time on the characteristics on microsphere entrapment

FORMULATION AND PROCESS VARIABLES	FORMULATION					
	MODIFIED EMULSIFICATION			COMPLEX COACERVATION		
	MT1	**MT2**	MT3	CT1	**CT2**	CT3
Drug:polymer ratio	1:4	**1:4**	1:4	1.4	**1.4**	1.4
Cross linker concentration (%)	7.5	**7.5**	7.5	1.5	**1.5**	1.5
Cross-linking time (min)	30	**45**	60	15	**30**	45
Entrapment efficiency (%)	69.23	**78.25**	63.45	67.19	**81.23**	71.21

Percentage yield

The yield of microspheres was determined by comparing the total weight of microspheres obtained against the sum of the weight of the drug, polymers and bioenhancer. The percentage yield was found to be 33.12 to 63.62% as shown in Table 6.

Bioadhesion study

The bioadhesion study was performed (in triplicate) using a previously reported method. The percentage bioadhesion was found to be 43.25 to 83.21% as shown in Table 6. The bioadhesive property (Fig. 3) of the microspheres in which bioenhancers were used is higher as compared to microspheres without bioenhancer. The bioadhesive property of microspheres resulted in prolonged retention of formulation in the small intestine. (Table 6).

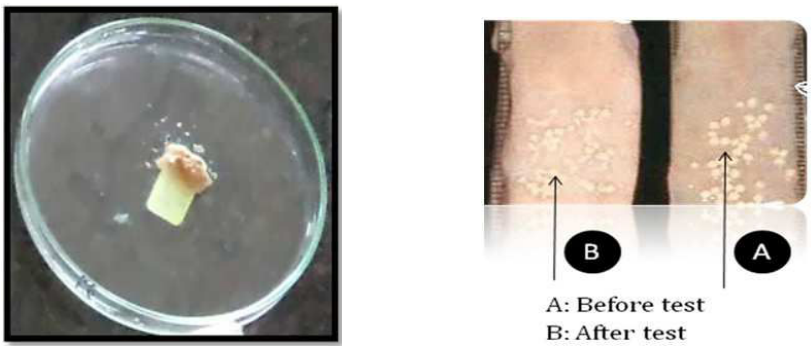


Figure 3.
Bioadhesion study for microspheres

Table 6
Particle size, yield, drug entrapment, bioadhesion and drug release of isoniazid microspheres by double emulsification and complex coacervation method

FORMULATION CODE	MEAN PARTICLE SIZE (µM)	YIELD (%)	DRUG ENTRAPMENT (%)	BIOADHESION (%) ± SD	DRUG RELEASE AT 12^{TH} HOUR
MF1	116-118	39.23 ± 2.23	64.54 ± 2.32	46.32 ± 1.21	43.25 ± 1.32
MF2	114-117	46.21 ± 1.98	71.45 ± 2.45	57.54 ± 1.97	58.23 ± 1.56
MF3	**109-112**	**63.62 ± 1.71**	**81.78 ± 2.12**	**83.21 ± 1.11**	**86.65 ± 2.12**
MF4	123-125	51.23 ± 2.34	76.34 ± 2.41	74.17 ± 2.54	67.42 ± 2.34
CF1	123-125	33.12 ± 2.98	61.56 ± 2.91	43.25 ± 2.67	41.67 ± 2.45
CF2	125-128	42.23 ± 2.65	66.67 ± 2.96	54.36 ± 2.23	53.47 ± 2.06
CF3	121-123	49.34 ± 2.12	71.54 ± 3.17	67.43 ± 1.34	65.68 ± 2.31
CF4	**118-121**	**60.28 ± 1.82**	**76.45 ± 2.01**	**81.23 ± 1.06**	**85.17 ± 1.72**

* ***Average of three formulations ± SD***

In vitro drug release

The in-vitro release behavior of isoniazid microspheres prepared by modified emulsification and complex coacervation method in simulated gastric fluid (SGF), pH 1.2 and simulated intestinal fluid (SIF), pH 6.8, is shown in Fig. 4a and 4b. Approximately 10-15% of the drug was released in the SGF, pH 1.2 over a period of 2 h and 35-75% in SIF, pH 6.8 up to 12 h. It has been found that the microspheres containing bioenhancer show a very high increase in the release of microspheres (43.25 to 86.65% and 41.67 to 85.17% in case of modified emulsification method and complex coacervation method respectively) as compared to microspheres without bioenhancer. In following figures, MF1 and CF1 are formulations without bioenhancer and MF2, MF3, MF4, CF2, CF3 and CF4 are the formulations where bioenhancer is used in 5, 10 and 15 mg respectively as shown in Table 6 and Figure 4a and 4b.

Figure 4a
***In-vitro* drug release of Isoniazid microsphere by modified emulsification method using *Piper nigrum* as a bioenhancer (BE)**

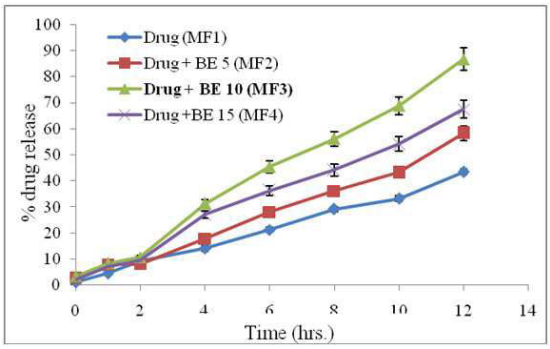
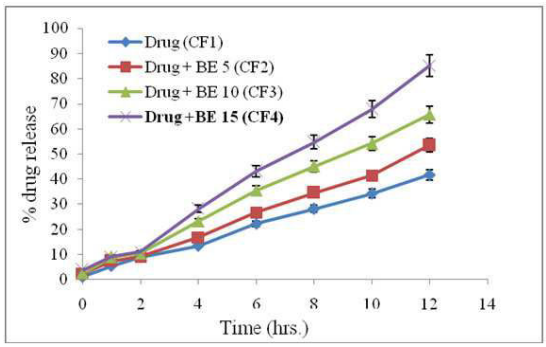


Figure 4b
***In-vitro* drug release of Isoniazid microsphere by complex coacervation method using *Piper nigrum* as a bioenhancer (BE)**



CONCLUSION

In the study, microspheres of isoniazid were prepared by double emulsification and complex coacervation method. Variables like drug-polymer ratio, cross linker concentration and the cross-linking time were considered for optimization of microspheres. FT-IR and DSC studies did not reveal any significant drug interactions with polymer and bioenhancers. Higher entrapment efficiency was obtained by double emulsification method (81.78%) as

compared to the complex coacervation method (76.45%). The drug release from the microspheres was affected by the pH of the dissolution medium. The presence of optimum concentration of bioenhancer was found to enhance the *in-vitro* release of the drug from 43.25% to 86.65% and from 41.67% to 85.17% for modified emulsification and complex coacervation method repsectively.

REFERENCES

1. Connell D.W., Berry M., Cooke G., Kon O.M., 2011. Update on Tuberculosis: TB in the early 21st Century. European Respiratory Review. 20 (120), 71-84
2. Sandhu G. K., 2011. Tuberculosis: Current Situation, Challenges and Overview of its Control Programs in India. Journal of Global Infectious Diseases. 3(2), 143-150.
3. Fauci A.S., 2001. Infectious Diseases: Considerations for the 21st Century. Clinical Infectious Diseases. 32 (5), 675-685.
4. Barrow E. L. W., Winchester G. A., Staas J. K., Quenelle D. C., Barrow W. W., 1998. Use of Microsphere Technology for Targeted Delivery of Rifampin to *Mycobacterium tuberculosis*-Infected Macrophages. Antimicrobial Agents and Chemotherapy. 42(10), 2682–2689.
5. Issar S., *2003. Mycobacterium tuberculosis* Pathogenesis and Molecular Determinants of Virulence. Clinical Microbiology Reviews. 16 (3), 463-496.
6. Toit L. C., Pillay V. Danckwerts M.P., 2006. Tuberculosis Chemotherapy: Current Drug Delivery Approaches. Respiratory Research. 7(118), 1-18.
7. Rastogi R., Sultana Y., Aqil M., Ali A., Kumar S., Chuttani K., Mishra A.K., 2007. Alginate Microspheres of Isoniazid for Oral Sustained Drug Delivery. International Journal of Pharmaceutics. 334, 71-77.
8. Randhawa G. K., Kullar J.S., Rajkumar., 2011. Bioenhancers from Mother Nature and Their Applicability in Modern Medicine. Inrternational Journal of Applied and Basic Medical Research. 1(1), 5-10.
9. Dudhatra G.B., Mody S.K., Awale M.M., Patel H.B., Modi C.M., Chauhan B.N., 2012. A Comprehensive Review on Pharmacotherapeutics of Herbal Bioenhancers. The Scientific World Journal. Article ID 637953, 1-33.
10. Atal N., Bedi K.L. 2010. Bioenhancers: A Revolutionary Concept to Market. Journal of Ayurveda and Integrative Medicine. 1(2), 96-99.
11. Patil U.K., Singh A., Chakraborty A. K., 2011. Role of Piperine as a Bioavailability Enhancer. International Journal of Recent Advances in Pharmaceutical Research. 4, 16-23.
12. Akhand K., Samnani A., Panday G., Dubey B.K., 2012. Microspheres as a Novel Drug Delivery Sysytem: A Review. World Journal of Pharmacy and Pharmaceutical Sciences. 1(1), 34-44.
13. Qazi G.N., Bedi K.L., Johri R.K., Tikoo M.K., Tikoo A.K., Sharma S.C., 2005. Bioavailability Enhancing Activity of *Carum carvi* Extract and Fractions Thereof. U.S. Patent. US 0214390 A1.
14. Kokate C.K., Purohit A.P., Gokhale S.B., 2002. Pharmacognosy. 46th edition. Nirali Prakashan, Pune. 1.55-1.57.
15. The Ayurvedic Pharmacopoeia of India. Department of Ayush, Ministry of Health and Family Welfare, Government of India. Part I Volume II. 141.
16. Raman G., Gaikar V.G., 2002. Extraction of Piperine from *Piper nigrum* (Black Pepper) by Hydrotropic Solubilization. Industrial and Engineering Chemistry Research. 41. 2966-2976.
17. Lucinda-Silva R. M., Evangelista R. C., 2003. Microspheres of Alginate - Chitosan Containing Isoniazid. Journal of Microencapsulation. 20(2), 145-52.
18. Ranga Rao K.V., Buri P., 1989. A Novel *in situ* Method to Test Polymers and Coated

Microparticles for Bioadhesion. International Journal of Pharmaceutics. 52, 265–270.

19. Phutane P., Shidhaye S., Lotlikar V., Ghule A., Sutar S., Kadam V., 2010. *In vitro* Evaluation of Novel Sustained Release Microspheres of Glipizide Prepared by the Emulsion Solvent Diffusion-Evaporation Method. Journal of Young Pharmacists. 2(1), 35-41.
20. Dinarvand R., Rahmani *E.,* Farbod E., 2003. Gelatin Microspheres for the Controlled Release of All-trans-Retinoic Acid Topical Formulation and Drug Delivery Evaluation. Iranian Journal of Pharmaceutical Research. 2. 47-50.