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Effect of Process Variables and Co-administration of Bioenhancer on *In-Vitro* Release of Rifampicin from oral Microspheres

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ABSTRACT

The major problems associated with the anti-tubercular (TB) drug therapy include loss of efficacy through bacterial resistance, side effects, low patient compliance and duration and complexity of treatment. The present study attempts to confront them through a combined approach consisting of microspheres and bioenhancers. Microspheres containing rifampicin were prepared by emulsification technique using stearic acid as a cross-linking agent. Extract of *Carum carvi* were added as a bioenhancer in variable amount of 5 to 15 mg for each dose of rifampicin. The loading efficiency and release behavior of loaded microparticles were found to be dependent on the cross-linker concentration, cross-linking time and drug-polymer ratio. Prolonged release of the drug from the microspheres was demonstrated in a simulated intestinal fluid. *In-vitro* release of rifampicin from the microspheres containing 15 mg of bioenhancer showed significant increase in release profile (87.42% in formulation containing bioenhancer against 51.41% for the formulation without bioenhancer) and the release rates were reduced upon increasing the amount of cross-liking agent and prolonging the cross-linking time.

Keywords: microspheres, rifampicin, sodium alginate, bioenhancer, *Carum carvi*.

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INTRODUCTION

Tuberculosis (TB), an infectious disease caused by the *Mycobacterium tuberculosis*, has afflicted the human race for centuries. Tuberculosis results in an estimated 1·7 million deaths each year and the worldwide number of new cases (more than 9 million) is higher than at any other time in history. 22 low-income and middle-income countries account for more than 80% of the active cases in the world.[1,2] The clinical management of the disease is limited because of the problems associated with the anti-TB drugs, such as their toxic effects, degradation before reaching their target site, low permeability, and bacterial resistance, ultimately resulting in poor patient compliance.[3]

Hence, in the development of effective anti- TB therapy, two points are very important. First, the metabolism of *M. tuberculosis* is slow, resulting in a generation time that is measured in hours. Second, the tubercle bacillus is a facultative intracellular parasite. 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 long term, but also would be able to target drugs to the intracellular environment in which the tubercle bacilli are found i.e., macrophages.[4]

Poor patient compliance is the most common reason for the failure of tuberculosis chemotherapy. One useful method to ensure compliance in tuberculosis patients is to administer the drugs in delivery systems that release drugs in a sustained manner at therapeutic concentrations over a period of time. This strategy helps to improve patient compliance in terms of reducing the dosage frequency, and may also minimize the risk of emergence of drug-resistant mutants and potential toxicity.[3, 5, 6]

Microsphere technology is one of the established techniques for controlled delivery used to deliver several different types of drugs, such as steroids, proteins and antibiotics.[4]

Rifampicin (RIF) is one of four drugs taken as part of a standard treatment regimen for TB. Discovered in 1957, it remains one of the strongest medications available for TB treatment. It inhibits DNA-dependent RNA polymerase, thus inhibiting transcription to RNA, making the bacterium unable to produce important proteins. Its biological half-life varies from 1.5 to 5 h.

A bioenhancer is an agent capable of enhancing bioavailability and efficacy of a drug with which it is co-administered, without any pharmacological activity of its own, at therapeutic dose used.[7]

Caraway (*Carum carvi*), also known as meridian fennel, is a biennial plant in the family *Apiceae*, native to western Asia, Europe and North Africa. It has carminative, mild stomachic,

aromatic, and diuretic actions. However, its extract has shown potent bioenhancer activity in various drug treatments, alone and in combination with other bioenhancers.[8] It enhances the bioavailability of antibiotics, antifungal, antiviral and anticancerous drugs. The effective dose of the bioenhancer extract is in the range of 5–100 mg/kg body weight and the dose of the bioactive fraction of bioenhancer is in the range of 1–55 mg/kg body weight. It shows greater bioenhancing effect when used in combination with other bioenhancers such as *Zingiber officinale* and piperine.[9,10]

The stearic acid is replaced with calcium chloride as a cross linking agent in preparation of microspheres. Dried microspheres were loaded by immersing them in an aqueous solution of rifampicin.[11] In order to prepare microspheres with an appropriate drug release profile, the effect of time of cross-linking and the amount of cross-linking agent on the swelling properties of microspheres and their release profile were investigated.

In this study, we have attempted a combined approach consisting of microspheres and bioenhancer in order to overcome the problems associated with the anti-TB drug therapy.

MATERIALS AND METHODS:

Rifampicin was obtained as a gift sample from Lupin Pharmaceuticals Ltd., Aurangabad, Maharashtra. Caraway (*Carum carvi*) was purchased from local market and authenticated from Dept. of Botany, Dr. P. R. Ghogrey College of Science, Dhule, MH. Sodium alginate was obtained from Loba Chemie, Mumbai. All other chemicals / polymers were of analytical grade.

Evaluation and Extraction of *Carum carvi* used as a bioenhancer:

Macroscopy of *Carum carvi*:

Fruits, greenish-brown, slightly curved, elongated, mericarps, usually separate, free from the pedicel, carpophores, upto 7 mm long, 2 mm broad almost equally five sided, narrow, tapering to each end, arcuate, glabrous, brown with five very narrow, yellowish primary ridges with characteristic odour and taste [12,13] figure 1.

Figure 1: *Carum carvi* fruits and powder

Phytochemical evaluation of *Carum carvi*:

Phytochemical evaluation of fully mature, fruits of *Carum carvi* Linn, was carried out as per Ayurvedic Pharmacopoeia of India for various parameters[13], the results were mentioned in Table

Table 1: Phytochemical evaluation of Caraway

Parameter	Results	Limit
Loss on drying	1.14 % w/w	Not more than 2 %w/w
Total ash	5.87 % w/w	Not more than 9.0% w/w
Acid insoluble ash	0.57 % w/w	Not more than 1.5% w/w
Water soluble extractive	16.11 % w/w	Not less than 12% w/w
Alcohol soluble extractive	7.53 % w/w	Not less than 2%w/w

Extraction of *Carum carvi*[14]:

C. carvi fruits (100 g) were air-dried and finely powdered, soaked in enough volume (500 ml) of ethanol: water (70:30) for 2 hours and extracted using percolation apparatus, which was maintained for 48 h to accomplish complete extraction. The extracted liquid was shaken, filtered and evaporated under reduced pressure until a semisolid extract was obtained. The extract was freeze-dried to obtain a dry powdered extract.

Preparation of rifampicin microspheres:[15,16,17]

Rifampicin (100 mg) was dispersed in 5% 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 1400 - 1600 rpm for 20 minutes. To the formed emulsion, 10 ml of 5% stearic acid dissolved in a mixture of ethanol and ethyl acetate (2:1) was added slowly and stirred to assure efficient cross-linking. Microspheres were collected by filtration in vacuum, washed with distilled water thrice and dried at room temperature. Small quantity (2-3 ml) of Isopropyl alcohol was added for dehydration of the microspheres. The formulations were prepared by varying the concentration of sodium alginate, ratio of sodium alginate to rifampicin, ratio of stearic acid, and the cross-linking time. Finally, the bio-enhancer extract was added to the optimized formulation in different concentrations to study its effect on the *in-vitro* release of rifampicin. The formulae of optimized formulations are shown in Table 2.

Table 2: Optimized formulae of Rifampicin microspheres

Formulation code	RIF (mg)	Drug:Polymer ratio	Stearic acid (%)	Cross linking time (min.)	*Carum carvi* extract(mg)
RF1	100	1:5	5	20	--
RF2	100	1:5	5	20	5
RF3	100	1:5	5	20	10
RF4	100	1:5	5	20	15

CHARACTERIZATION OF THE MICROSPHERES:

Compatibility studies:

Chemical interaction between the drug and the polymer material, if any, during the preparation of the microspheres was studied by using Fourier Transform Infrared Spectroscopy (FTIR). Pure drug, placebo microspheres, microspheres (10 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 spectrums of the pellets 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 = 327 nm; Perkin Elmer, USA Lambda 25 model). Microspheres (10 mg) loaded with rifampicin 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 filter and the amount of rifampicin was determined. Preliminary UV studies showed that the presence of dissolved polymers did not interfere with the absorbance of the drug at 327 nm.

The percent drug entrapment was calculated using following formula:

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

Percentage yield:

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

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

Measurement of bioadhesion:

In-vitro bioadhesion was determined for microspheres (in triplicate) by falling liquid film

method. 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.[20] Percent bioadhesion was determined by the following formula:

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

In-vitro drug release:

Dissolution studies were carried out using USP XXIV rotating basket method. The release profiles of rifampicin 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 20 mg of rifampicin) filled in empty capsule shells were put into the basket (50 rpm) and placed in 500 ml of the dissolution medium, thermostated at 37^{0}C. Samples of 2 ml each were withdrawn at regular time intervals, filtered, diluted suitably, analyzed using double beam UV spectrophotometer at 475 nm and an equal volume of fresh medium was immediately added to maintain the dissolution volume[21]. Dissolution studies were carried out up to 12 h. The drug release experiments were conducted in triplicate.

RESULTS AND DISCUSSION:

Compatibility studies:

After performing the compatibility studies, it was found that rifampicin is compatible with different excipients, polymers and blends of bioenhancer using FTIR figure 2a, 2b, and 2c.

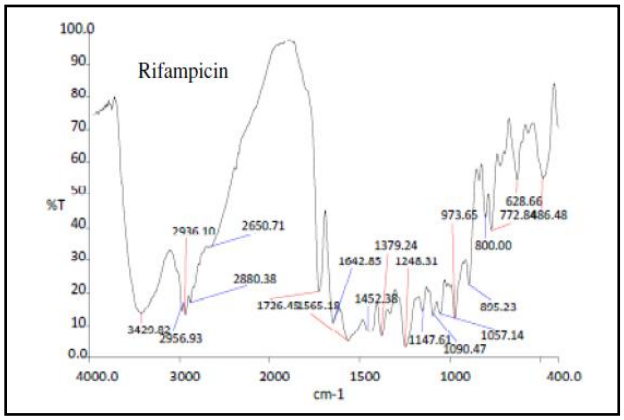

Figure 2a: IR spectra of rifampicin

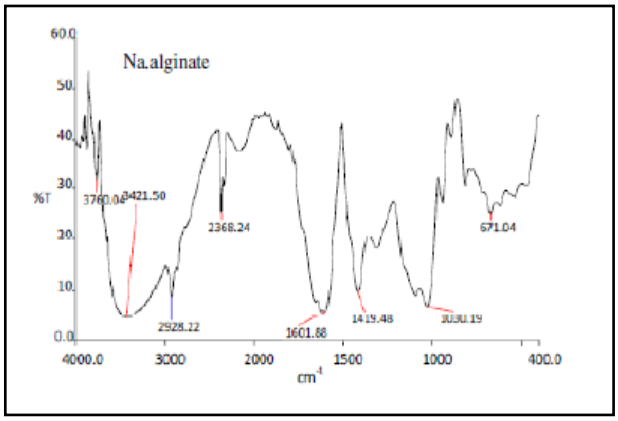

Figure 2b: IR spectra of sodium alginate

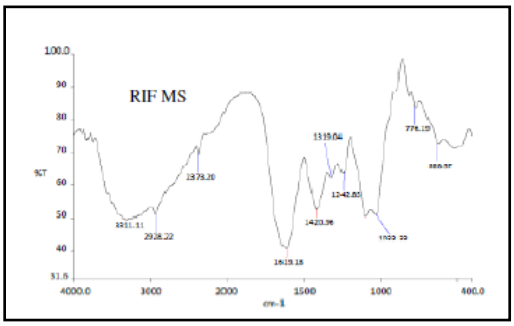

Figure 2c: IR spectra of rifampicin microspheres

Particle size analysis:

The microspheres had a smoother surface and were found to be discrete and spherical in shape as shown in Figure 3. The use of isopropyl alcohol resulted in dehydration of the particles with decrease in their particle size.[18]

No change in the morphology was observed in drug-loaded microspheres. The mean particle size of the microspheres without bioenhancer and with bioenhancer was found to decrease steadily with increasing concentration of bioenhancer, from 121-134 µ (without bioenhancer) to 103-112 µ (maximum concentration of bioenhancer) as shown in Table 3.

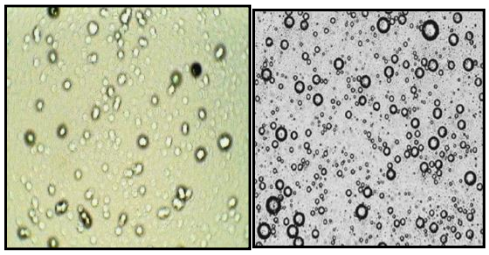

Figure 3: Rifampicin microspheres by emulsification method

Determination of percentage drug entrapment:

The % entrapment efficiency was found to be in the range of 36.13 – 85.97, as shown in Tables 3 and 4. The maximum values obtained are higher, or at least comparable to the highest value of entrapment efficiency reported in earlier studies using the sodium alginate method of preparation of microspheres.[19]

The difference is due to the high aqueous solubility of RIF resulting in high concentrations of the drug present in the preparation medium in this method and possibly due to the use of bioenhancer in formulations.

Percentage yield:

The percentage yield of optimized formulations was found to be 31.50 - 67.10% as shown in Table 3. The loss of the drug in the method may be due to loss accounted during hardening, washing and filtering processes of microspheres.

Bioadhesion study:

The bioadhesion of the microspheres in the optimized formulations showed a significant change with the presence and quantity of bioenhancer. The microsphere without bioenhancer has 49.25% bioadhesion, while the microspheres containing comparably higher amount of bioenhancer showed significant increase in bioadhesion about 86%. It has also been observed that, the percentage bioadhesion increases as the amount of bioenhancer increases (Table 3). The bioadhesive property of these particles resulted in prolonged retention in the small intestine.

Table 3: Particle size, yield, drug entrapment, bioadhesion and drug release of rifampicin microspheres

Formulation code	Mean particle size (µm)	Yield (%)	Drug Entrapment (%)	Bioadhesion (%)± SD	Drug release at 12th hour
RF1	121-134	31.50 ± 1.88	35.89 ± 1.32	49.25±1.68	51.41 ± 1.81
RF2	118-123	45.63 ± 2.18	47.45 ± 2.08	61.23 ± 1.78	59.17 ± 1.64
RF3	114-127	64.56 ± 1.89	63.78 ± 2.17	67.14 ± 1.71	66.43 ± 2.16
RF4	103-112	67.10 ± 1.34	85.87 ± 1.75	82.45±1.19	87.42 ±1.51

In-vitro drug release:

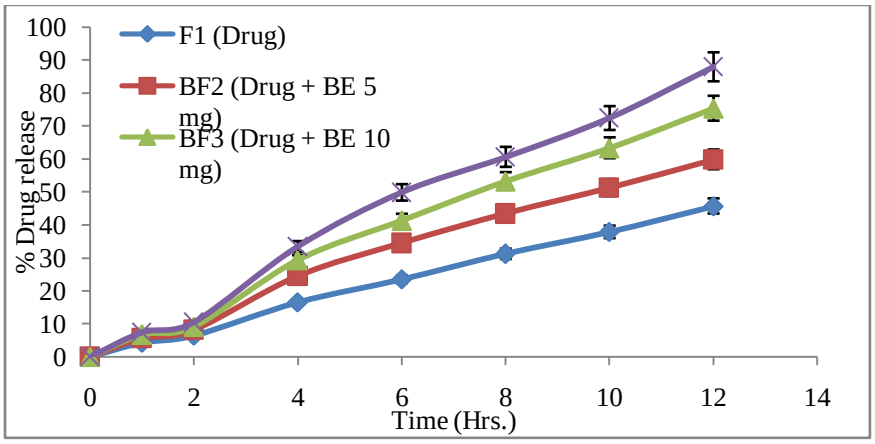

Figure 4: Drug release profile of optimized formulation of rifampicin microspheres in SGF pH 1.2 and SIF pH 7.4.

The *in-vitro* release behavior of rifampicin microspheres prepared by emulsification method using stearic acid as a cross-liking agent in simulated gastric fluid (SGF), pH 1.2 and simulated intestinal fluid (SIF), pH 7.4, is shown in Figure 4. Approximately, 6-10% of the drug was released in the SGF, pH 1.2 over a period of 2 h and about 45-78% in SIF, pH 7.4 up to 12 h. It has been found that the microspheres containing bioenhancer show greater increase in drug release as compared to microspheres without bioenhancer. In figure 4, RF1-formulations without bioenhancer and RF2, RF3 and RF4 are the formulations where *Carum carvi* used as bioenhancer in 5, 10 and 15 mg respectively as shown in Table 3.

Effect of cross-linker concentration:

Concentration of the cross-linker up to 5% caused increase in the entrapment efficiency and the extent of drug release (Figure 5a). Nevertheless, more increase in stearic acid concentration resulted in increase in the particle size and decrease in the entrapment efficiency as shown in table 4. It is probably because Rifampicin being highly soluble in water comes out of the gel along with the squeezed aqueous phase.

Effect of drug–polymer ratio:

The effect of drug–polymer ratio on RIF release from different batches of microspheres is shown in Figure 5b. Though the release profile was found to be controlled in all the cases, drug-polymer ratio of 1:5 resulted in the highest concentration of RIF in the selected media. A reduced drug release rate was observed with relative increase in the polymer concentration in microspheres. This can be characterized to the increase in the thickness of the polymer matrix with increased polymer concentration (1:10). While, drug polymer ratios below 1:5 showed low entrapment efficiency (Table 4) due to insufficient concentration of the polymer.

Table 4: Effect of drug: polymer ratio on rifampicin microspheres

Formulation variables	Formulations				
	F1	F2	F3	F4	F5
Drug:polymer ratio	1:2	1:5	1:5	1:10	1:10
Cross-linker concentration (%)	3	3	5	5	7
Entrapment efficiency (%)	46.43	49.21	78.32	71.21	56.23

Effect of cross-linking time:

Variations in the cross-linking time were also studied for selecting the best – optimized formulation. Cross-linking time of 20 minutes was found to be sufficient for better drug entrapment efficiency (Table 5); less cross-linking time resulted in incomplete gelling of sodium alginate. Increasing the cross-linking time greater than 20 minutes upto 30 and 45 minutes however, caused no significant change in the drug release (Figure 5c).

Table 5: Effect of cross-linking time on the entrapment efficiency

Formulation and process variables	Formulation		
	C1	C2	C3
Drug: polymer ratio	1:5	**1:5**	1:5
Cross-linker concentration (%)	5	**5**	5
Cross-linking time (min)	10	**20**	30
Entrapment efficiency (%)	36.13	**85.97**	72.31

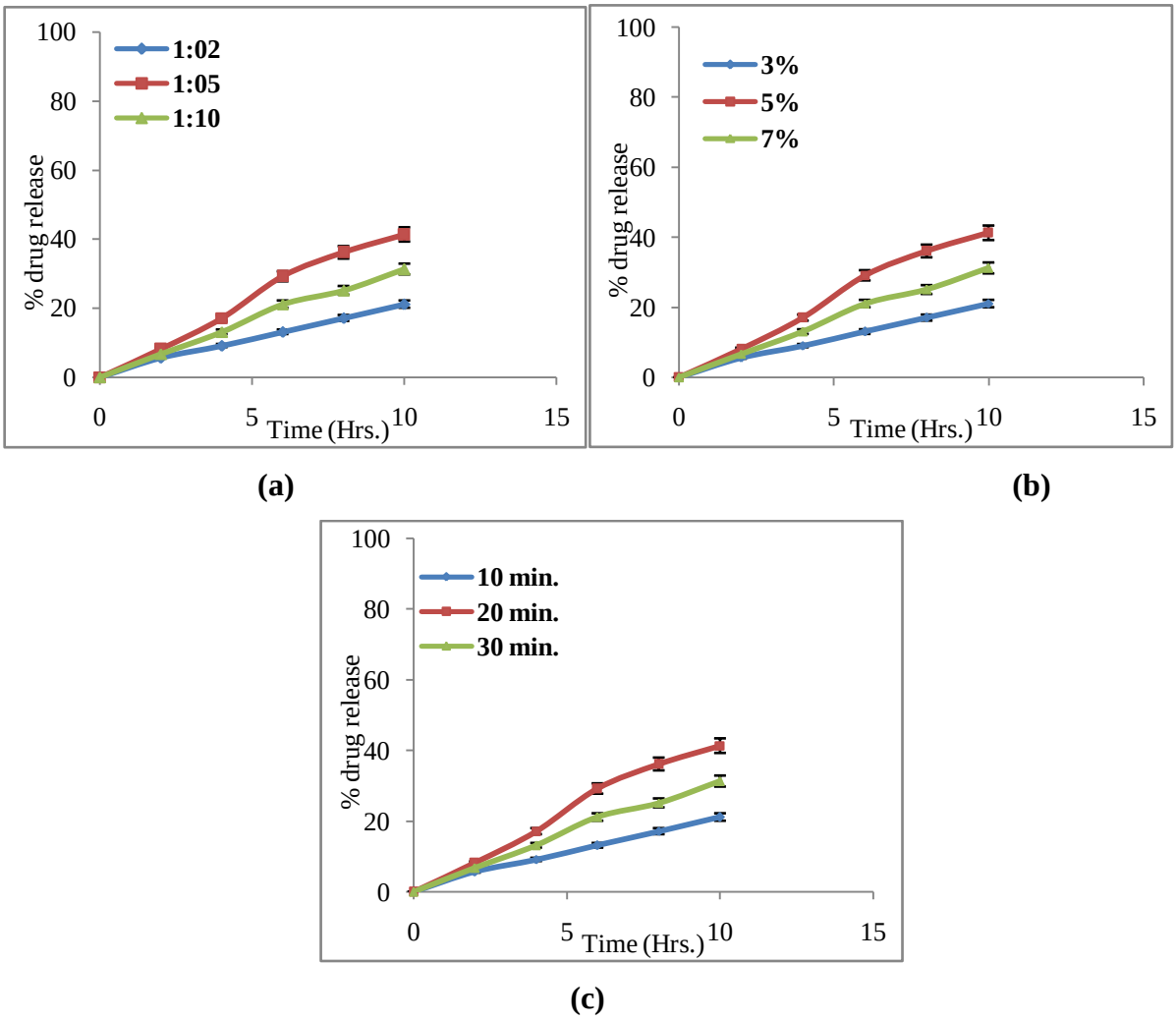

Figure 5: The release profiles of rifampicin from microspheres in SIF pH 7.4 under variable; cross-linker concentration(a), drug: polymer ratios(b) and cross-linking times(c).

CONCLUSION:

In this study, microspheres of rifampicin were prepared by emulsification method using sodium alginate as a polymer and stearic acid as a cross-linking agent. Effect of variables like drug-polymer ratio, cross linker concentration and the cross-linking time on *in-vitro* release of rifampicin was examined. The mean particle size of the microspheres increased with an increase in the concentration of polymer. The increase in the concentration of stearic acid and sodium alginate caused the increase in the entrapment efficiency and the extent of drug release. The cross-linking time shorter than 20 minutes resulted in higher entrapment efficiencies. The microspheres were spherical and well formed. The mean diameter, entrapment efficiency and bioadhesion of the optimized microspheres were found to be 103 -112 µm, 85.87 ± 1.75% and 87.42 ±1.51% respectively. The release profiles of rifampicin from microspheres were examined

in simulated gastric fluid (SGF pH 1.2) and simulated intestinal fluid (SIF pH 7.4). About 10% of the rifampicin was released in the SGF in first 2 hours and released quickly about 85% in 10 hrs in SIF. The concentration of sodium alginate and the presence of stearic acid had great affect on the release of rifampicin. The most important finding of this study relates to the very significant enhancement in drug release (51.41 to 87.42%), due to co-administration of 15 mg bioenhancer along with each dose of rifampicin microspheres.

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