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FORMULATION, CHARACTERIZATION AND EVALUATION OF MICROSPHERES CONTAINING ISONIAZID

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ABSTRACT

The present study was aimed to develop and evaluate microspheres of isonicotinylhydrazide (INH) in different drug to polymer ratios using emulsion solvent Evaporation method.[i] FTIR studies showed that there was no chemical interaction between the drug and polymers. Scanning electron microscopy showed the microspheres having a spherical structure. Prepared microspheres were characterized for calibration curve, FTIR, % drug loading and *in-vitro* drug release studies. In-vitro drug release studies were performed using the shaking flask method. The aim of present work is to investigate the possibility of obtaining a prolonged, reducing the side effects, increase the patient compliance, relatively constant effect of isoniazid microspheres by using Carbopol as carrier.[ii]

INTRODUCTION

Isoniazid is a first line antitubercular drug. It has broad spectrum antimicrobial activity. They act as both bacteriostatics for resting bacilli and bacteriocidal for dividing microorganisms. Isoniazid is a prodrug i.e. converted by enzyme known as mycobacterial catalase peroxidase into an active metabolite. Mycolic acid is a unique fatty acid component of mycobacterial cell wall.[iii] Isoniazid is inhibits the biosynthesis of mycolic acid in bacterial cell wall. Isoniazid acts on enoyl-ACP reductase of fatty acid synthase-II, cause saturation of fatty acid in mycolic acid biosynthesis .Isoniazid is absorbed by oral and parental administration. Metabolized by liver, and excreted in the urine, t1/2 is about 3 hours. Dose 5mg/kg (adult dose = 300mg / day).[iv] Aluminum hydroxide inhibits the absorption of isoniazid. Para-amino salicylic acid inhibits the metabolism of isoniazid and increase t1/2 of isoniazid. Isoniazid inhibit metabolism of warfarin, phenytoin, carbamazepine and diazepam which may raise their concentration in blood. Peripheral neutritis, neurological manifestations, hepatotoxicity, rashes, fever, acne and arthralgia etc. are common side effects[v]. Microencapsulation is the protective technology of encapsulating solid, liquid or gas materials into micro particles with a diameter of 1–1000 μm, and has been widely used in fields of medicine, cosmetics, food, textile and advanced materials[vi]. The unique advantage of microencapsulation lies in that the core material is completely coated and isolated from external environment. More importantly, microencapsulation would not affect the properties of core materials, provided that proper shell material and preparing method are chosen[vii] . Microspheres offer a number of advantages in therapeutics. Microspheres are of the drug delivery system which provide programmed and controlled release drug after proper duration of action at particular site[viii].

Carbopol polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. They are primary produced from polymer having particles of about 0.2 to 6.0 micron in diameter. Carbomer-934 is to be a polymer of acrylic acid cross-linked with alkyl-sucrose: CH = CHCH2-O-sucrose.Carbomer-934P is the pharmaceutical grade of Carbomer-934 (Lubrizol, 2006)[ix].

MATERIALS AND METHDS:

Isoniazid gift sample from LABO CHEM; Carbopol; Chloroform, Sodium CMC,Tween 80, Double beam UV spectrophotometer, Beakers, Magnetic stirrer, Mechanical stirrer, Orbital shaker.

Identification test: 100mg of isoniazid was dissolved in 2mL of Distilled water, added 10mL warmed solution of vanillin (1%, in water). Kept for aside for some minutes, after that scratch the inside of container with glass rod, yellow precipitate was obtained. Precipitate was recrystallized with 70% ethanol (5mL) and dried at 105oC. Yellow precipitates melt at 226oC -231oC[x].

Solubility: Isoniazid is most soluble in methanol, followed by acetone, ethanol and ethyl acetate at a temperature up to 308 k. At higher than 308k; isoniazid is most soluble in acetone[xi].

METHOD OF PREPARATION

CALIBRATION CURVE: Isoniazid stock solution was prepared by accurately weighing 100mg of pure sample and Dissolving in distilled water in a 100ml volumetric flask and made up to volume. Several volumes of isoniazid corresponding to 0.1- 0.5mg were obtained upon dilution of the stock solution. This was then transferred to 25ml volumetric flasks. 4ml of vanillin solution was added followed by addition of 0.5M ethanolic hydrochloric acid and then thorough mixing. It was left to stand for full colour development for 10minutes before taking absorbance reading of the solutions. This experiment was repeated on 3 separate days and the mean values taken. From this, calibration curve was plotted at 360nm and regression analysis carried out. The calibration curve was validated by following the same procedure but changing the serial concentrations. The average absorbance readings were correlated directly with the concentrations using the calibration curves.

FTIR: Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time[xii].

Determination of % yield of microspheres: The dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formula given below.

% Yield = (Mass of micro-spheres obtained / Total weight of drug and polymer)*100

Bulk density: In this method microspheres are transferred to a measuring cylinder and are tapped till a constant volume obtained. This volume is bulk volume and it includes true volume of the powder[xiii].

Tapped density: The tapped density is measured by transferring the microspheres in the cylinder and fix to the tap density apparatus and the tapping is performed about 100 times. Then, Carss's compressibility index and Hasuner ratio were determined using the following equations[xiv]: Carr's compressibility index (%) = {Tapped density-bulk density X 100}/ Tapped density

Hausner ratio = [Tapped density / bulk density] X 100

Angle of repose: Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on to the surface. The height and radius of the powder cone was measured and. Angle of repose was calculated[xv].

Angle of repose (θ) = $\tan^{-1}$ (h/r)

SNO	CONCENTRATION	ABSORBANCE
1	0.5	0.085
2	1	0.149
3	2	0.207
4	3	0.341
5	4	0.433
6	5	0.510

Table 1: Table for absorbance of serial dilution.

CALIBRATION CURVE OF ISONIAZID API

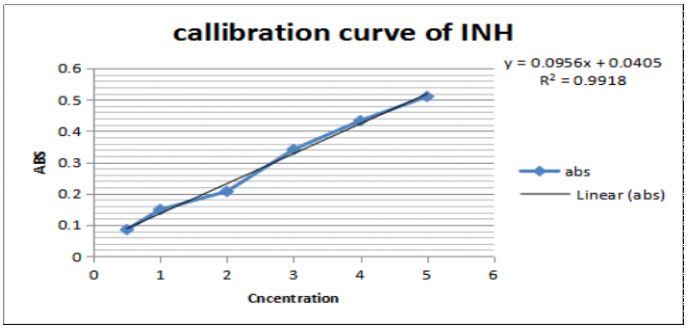


Figure 1: Calibration Curve of Isoniazid API

Microcapsules of isoniazid were compared with pure drug by FTIR. FTIR shows that drug and Polymer is compatible.

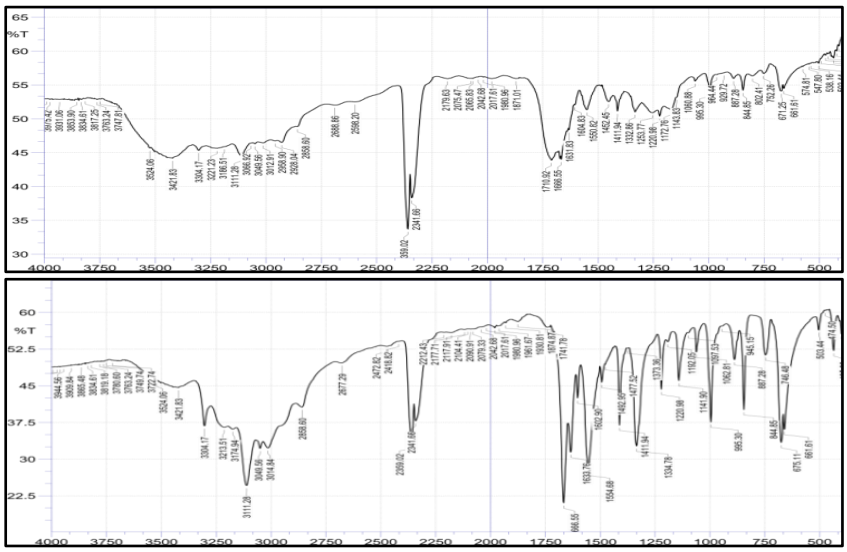


Figure2& 2: FTIR of drug and polymer

% Yield of INH microspheres: The % of the yield is show maximum in case of 1:3 drug polymer ratios in INH3.

Formulation code	Drug: polymer	% practical yield
INH1	1:1	59.74±0.24
INH2	1:2	65.12±0.81
INH3	1:3	74.46±0.62

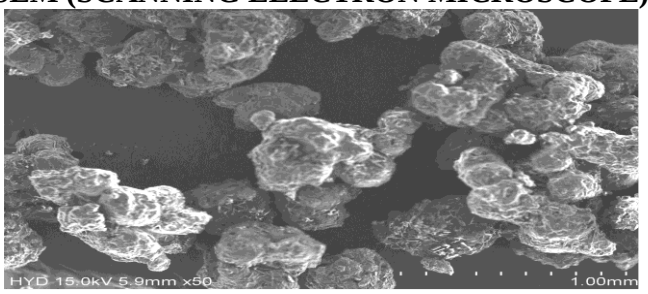
Table 1: % practical yield of INH microspheres

INH3 had highest density and lowest angle of repose which can conclude the highest flow property of INH3.

code	Bulk. density (gm./cm3)	Tapped density (gm./cm3)	Hausener's ratio	Carr's index	Angle of repose
INH1	0.30±0.01	0.31 ± 0.01	1.03 ± 0.01	36.12± 0.01	21.17±0.12
INH2	0.38 ±0.01	0.39 ± 0.01	1.03 ± 0.01	34.29 ± 0.02	16.90±0.13
INH3	0.41±0.02	0.43± 0.02	1.03 ± 0.01	32.58 ± 0.02	14.92±0.11

Table 2: Micromeritics of INH microspheres

SEM (SCANNING ELECTRON MICROSCOPE)



SEM photos of INH Microspheres

Scanning electron microscope sowed perfect shape and size of microsphere. Carbopol polymer is a suitable macromolecule for the preparation of microspheres of isoniazid. SEM studies showed that particles are spherical shape.

***INVITRO* RELEASE PROFILE OF MICROSPHERES**

Time (hrs.)	Absorbance	Concentration	Amount present	% released
0.5	1.03	10.35	10.35	10.35
1	2.11	21.34	21.34	21.34
2	2.78	29.15	29.15	29.15
3	3.68	37.25	37.25	37.25
4	4.75	47.97	47.97	47.97
5	5.25	53.03	53.03	53.03
6	6.80	68.8	68.8	68.8
7	7.15	72.22	72.22	72.22
8	7.93	80.13	80.13	80.13
9	8.56	86.46	86.46	86.46
10	9.22	93.13	93.13	93.13

Table 3: *In vitro* Release Profile of Microspheres (INH-1) of Isoniazid

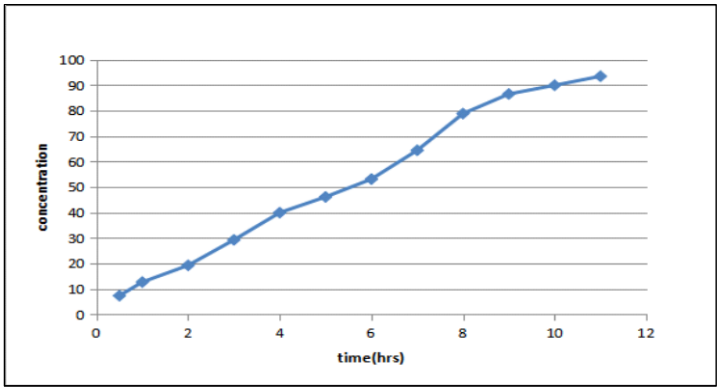


Figure 3: *In vitro* release graph of microspheres (INH-1) of isoniazid

Time (hrs.)	Absorbence	Concentration	Amount present	% released
0.5	0.95	9.5	9.5	9.5
1	1.75	17.67	17.67	17.67
2	2.10	21.21	21.21	21.21
3	3.05	30.8	30.8	30.8
4	4.25	42.9	42.9	42.9
5	4.98	50.36	50.36	50.36
6	5.60	56.56	56.56	56.56
7	6.92	69.89	69.89	69.89
8	8.13	82.12	82.12	82.12
9	8.70	87.87	87.87	87.87
10	9.05	91.41	91.41	91.41

Table 4: *In vitro* Release Profile of Microspheres (INH-2) of Isoniazid

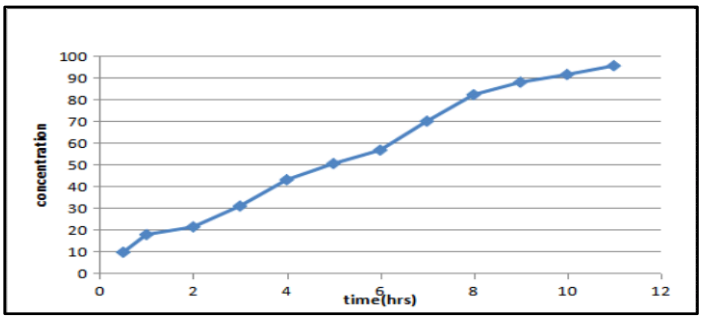


Figure 4: In vitro release graph of microspheres (INH-2) of isoniazid

Time (hrs.)	Absorbance	Concentration	Amount present	% released
0.5	0.72	7.27	7.27	7.27
1	1.25	12.62	12.62	12.62
2	1.90	19.19	19.19	19.19
3	2.89	29.19	29.19	29.19
4	3.95	39.89	39.89	39.89
5	4.56	46.06	46.06	46.06
6	5.25	53.04	53.04	53.04
7	6.37	64.34	64.34	64.34
8	7.80	78.78	78.78	78.78
9	8.56	86.46	86.46	86.46
10	8.90	89.89	89.89	89.89

Table 5: *In vitro* Release Profile of Microspheres (INH -3)

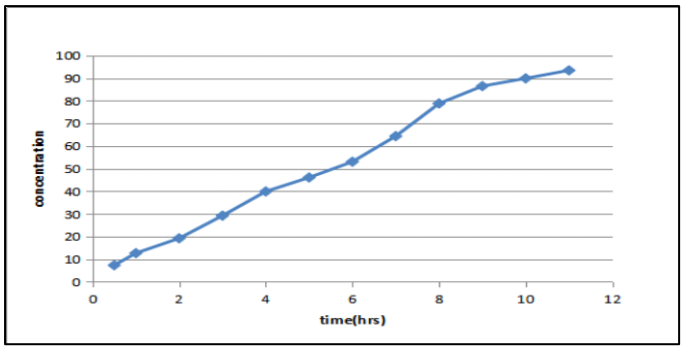


Figure 5: *In vitro* release graph of microspheres (INH-3) of isoniazid

Preparation of micro-spheres of isoniazid by emulsion solvent evaporation technique method:

Microsphere formulations using carbopol as a carrier polymer were prepared using emulsion solvent evaporation technique. Desired quantity carbopol polymer was dissolved in 10 ml of chloroform to form a homogenous polymer solution.[xvi] Calculated quantity of drug was added to this polymer solution and mixed thoroughly. The resulting mixture was then added to 250 ml of aqueous mucilage of sodium CMC (0.5%) containing 1% v/v tween 80, while stirring at 1000 rpm for emulsification. Chloroform was removed by evaporation during continuous stirring at room temperature for 3 h to produce spherical microspheres. Microspheres were collected by vacuum filtration, washed repeatedly with distilled water and petroleum ether and dried at room temperature for 24 h to get free flowing microspheres. The obtained microspheres were then vacuum dried in a decicator overnight[xvii]

***IN VITRO* DRUG RELEASE STUDIES**

Shaking flask method: Drug loaded microspheres equivalent to 100 mg of drug were weighed and transferred into a 100 ml conical flask. To this 100ml of pH 7.4 phosphate buffer saline was added, then the flasks were kept in a metabolic shaker and the shaker was adjusted to 50 horizontal shakes per minutes at 37 ± 0.5oC. One ml of the drug releasing media was withdrawn at various time interval of 30 min, 1, 2, 3, 4, 5, 6, 7, 8,9 and 10 hours and replaced by the same volume of phosphate buffer saline. These samples were filtered through 0.45 µm membrane filter. The filtrate was diluted suitably. The drug was estimate in each batch by UV spectrometer at 360 nm

RESULTS AND DISCUSSIONS: The entire calibration curve (Isoniazid) was found to obey the Beer-Lambert's law within the concentration range of 0.5-5 µg/ml. *in-vitro* drug release were based on these calibration curves.

CONCLUSION: Isoniazid Microsphere formulations using carbopol as a carrier polymer were prepared using emulsion solvent evaporation technique was successful. The ratio of drug and polymer 1:3 (INH 3) showed prolonged release and its flow property also increased. Microsphere resulted in significant improvement in physical properties of the drugs especially with respect to flow properties, bulk density and organoleptic properties. It's also resulted in delayed release of the drugs. The *in vitro* drug release studies indicated that the optimum release profile was found by formulation.

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