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MICROENCAPSULATION OF ISONIAZID BY TEMPERATURE CHANGE METHOD: PREPARATION AND CHARACTERIZATION

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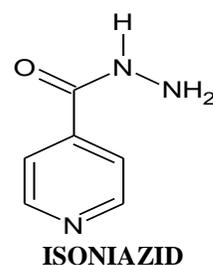
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ABSTRACT: Aim: Isoniazid is unstable in aqueous medium. The aim is to improve the aqueous stability of isoniazid by microencapsulation method. **Methods:** Isoniazid microcapsules were prepared by temperature change method. Ethyl cellulose used as coating material which forms a layer on pure drug. Ethyl cellulose was insoluble in cold cyclohexane when temperature rise about 70-75°C it become soluble in cyclohexane.

Result: Isoniazid was reported unstable in aqueous medium and their high acetylation occurs in liver. Microcapsules were prepared by temperature change method. The average particle size of isoniazid microcapsules was found to be 57µm and angle of repose was 23.56°, flow rate 0.85gm/sec. Isoniazid in pure form released 90% about in 50 minutes. After microencapsulation T90% was increased from 50 minutes to about 4 hours.

Conclusion: After microencapsulation improved the physical properties like bulk density, particle size, and flow characteristics. Isoniazid microcapsules also show prolonged the release by following first order kinetics and enhanced the stability in aqueous medium.

INTRODUCTION: Tuberculosis is a fatal communicable disease, caused by bacteria *Mycobacterium tuberculosis*, typically attacks the lungs but can also affect other part of the body, spread through air. About 1/3 of the world's population have infected by *Mycobacterium tuberculosis*. Due to TB about 2 million people will die yearly¹. WHO introduce the modern standard short course therapy for tuberculosis treatment based on four drug regimen of isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months followed by treatment with combination of isoniazid and rifampicin for 4 months².



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. Isoniazid 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 biosynthesis³.

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Isoniazid is absorbed by oral and parental administration. Metabolized by liver, and excreted in the urine, $t_{1/2}$ is about 3 hours. Dose 5mg/kg (adult dose = 300mg / day). Aluminum hydroxide inhibits the absorption of isoniazid. Para-amino salicylic acid inhibits the metabolism of isoniazid and increase $t_{1/2}$ of isoniazid. Isoniazid inhibit metabolism of warfarin, phenytoin, carbamazepine and diazepam which may raise their concentration in blood. Peripheral neuritis, neurological manifestations, hepatotoxicity, rashes, fever, acne and arthralgia etc. are common side effects ⁴.

Microencapsulation: Microencapsulation is a process in which small droplets or particles of liquid or solid material are surrounded or coated by a continuous film of polymeric materials.⁵ Microencapsulation is a techniques in which entrapping of solid/ liquid or gases by coating material, improving pharmaceutical application like masking unpleasant taste and odors, protecting drugs from moisture.⁶ Microencapsulation is occur by incorpora-tion of thin coating material on core material having particle size ranges from 5-5000 μ m. With the help of coating material, improve the availability and physiochemical properties of core material (drug). Microencapsulation increases the absorption of drug and suppress the side effect like irritation of GI mucosa ⁷. Microencapsulation has accepted for controlled release of drugs ⁸. It has overcome some of the problems of conventional therapy and increased the therapeutic efficacy of drug ⁹.

Objectives:

- The main reason for microencapsulation is for sustained or prolonged release of the drug.
- To improve the aqueous incompatibility.

MATERIALS AND METHODS: Double beam UV spectrophotometer, digital pH meter, hot magnetic stirrer, digital weighing balance, hot air oven, beaker, measuring cylinder, conical flask, stirring rod, funnel, methanol, cyclohexane, conc. HCl, Ethyl cellulose, microscope, capillary tube etc. Isoniazid pure drug obtained as a gift sample from Dr. Reddy's Laboratories Ltd.

Preformulation studies

- (A) **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 105°C. Yellow precipitates melt at 226°C -231°C.
- (B) **M.P. studies:** isoniazid melts at 170°C to 174°C.
- (C) **Solubility Analysis:** Isoniazid freely soluble in water, sparingly soluble in alcohol, very slightly soluble in ether ¹⁰.
- (D) **UV spectrophotometry studies:**

Preparation of 0.012M HCl solution: 0.012M HCl was prepared as per IP 1996.

Preparation of standard solution: 100 mg of Isoniazid was transferred to a 100mL volumetric flask containing sufficient quantity of the 0.012M HCl solution to dissolve it. The volume of solution was made up to 100mL with 0.012M HCl solution (1mg/mL).

Preparation of calibration curve of isoniazid: A series of working solution of Isoniazid ranging from 5 to 25 μ g/mL were prepared from standard solution. The absorbance of all solution was measured spectrophotometrically at 257 nm ¹¹.

Formulation design:

Preparation of Isoniazid Microcapsules by Temperature Change Method: Microcapsules were prepared a method described by ¹² with slight modification. 50mL cyclohexane was taken into a beaker with continue stirring at 200-400rpm and maintained the temperature at 55°C. After that 4g coating material (ethyl cellulose) was added into the solution. Increased the temperature of solution to 70°C for solubilization in the solvent for 20 minutes. 2g of isoniazid (core material) was added, maintained the temperature app. 70-75°C for 10 minutes.

Cool at room temperature for 5 minutes. Excess cooling was gained with the addition of ice (temp. 5-10°C). Filtered the sample by using Buchner funnel at the vacuum pump with Whatman filter paper No1. Microcapsules was rinsed with n-hexane and dried at 45°C for 3-4 hrs. Stored in well closed container for further studies (Bajaj et al., 2012).

EVALUATION OF MICROCAPSULES:

Color and Visual Appearance: Pure drug and microcapsules of isoniazid were visually seen for their overall appearance.

Particle Size Analysis by Microscopy: Particle size distribution plays a vital role in the evaluation of the release character of microcapsules. The sample of pure drug and prepared microcapsules was randomly selected and their size was determined using a simple microscopic method. About 100 microcapsules were counted for particle size analysis with the help of calibrated optical microscope^{13, 14}.

Moisture content determination: humidity (moisture) was determination on the basis of dry weight from a wet solid, called as moisture content (MC). Percent moisture content was determined by using following formula¹⁵;

$$\% \text{ MC} = \left[\frac{\text{wt. of water in sample}}{\text{wt. of dry sample}} \right] \times 100$$

Bulk density:

Apparent bulk density (g/mL): Accurately weighed quantity of microcapsules (5g) was taken into clean and dry graduated measuring cylinder. Note the volume of microcapsule in measuring cylinder. Bulk density was determined by using following formula⁷:

$$\text{Apparent bulk density} = \frac{\text{Weight of sample}}{\text{Volume of sample}}$$

Tapped density: Firstly set bulk densitometer for 100 strokes. Microcapsules (5g) was placed on bulk densitometer, after 100 tapping note the volume of microcapsules in cylinder. Tapped density was measured by using following formula¹⁶:

$$\text{Tapped density} = \frac{\text{Weight of sample}}{\text{Volume of sample after 100 tapping}}$$

Carr's index: it was calculated by using following formula^{17, 18}:

$$\% \text{ Carr's index} = \left(1 - \frac{\text{Poured Density}}{\text{Tapped Density}} \right) \times 100$$

Values of Carr's index less than 15% shows good flow personality, but more than 40% shows poor flowability.

Flow properties:

Angle of Repose: The flow property of microcapsules was determined by measuring angle of repose using fixed funnel method. Accurately weighed quantity of microcapsules (5g) was passed through funnel clamped on stand and measured the height and radius of the piles. Angle of repose was estimated by using following formula¹⁹:

$$\tan \theta = h/r$$

$$\theta = \tan^{-1} (h/r)$$

Where; θ = angle of repose, h = height of pile, r = radius of the base of the pile.

Flow rate: Accurately weighed quantities of microcapsules (5g) were taken and pass through a funnel clamp on stand. Time (in sec.) was noted required for falling of microcapsules from beginning to end by funnel orifice.

$$\text{Flow rate} = \frac{\text{weight of sample taken}}{\text{time in second}}$$

Coefficient of Friction: The tangents of angle of repose were defined as coefficient of interparticulate resistance or friction. If value of μ below 1 indicates good flow characteristics, but above 1 shows poor flow ability.

$$\mu = \tan \theta,$$

Where, μ = coefficient of friction and θ = angle of repose

Determination of pH: Isoniazid microcapsule was dissolved in distilled water, determined the pH with the help of pH meter.

Dissolution rate studies of Microcapsules:

Accurately weighed 100mg pure drug and 200mg microcapsules of isoniazid were filled in hard gelatin capsules. Each capsule was placed in USP XXII dissolution apparatus, the studies was carried out by using 900mL of pH 7.4 phosphate buffer at 100 rpm. 10mL sample was withdrawn at every half hour of time interval and replaced with same amount of pH 7.4 buffer to maintain the ideal sink conditions, which were further diluted with pH7.4 buffer. Absorbance was taken at 263 nm with the help of UV spectrophotometer ⁷.

Permeation test: Egg was buying from local market and removed the yolk, kept in HCl for 48 hrs. Egg membrane was leaving the wall of egg shell. Two both sided hollow test tubes were taken. One side test tube was wrapped by egg membrane. Took 100mg pure drug and 200mg microcapsules of isoniazid into these test tubes and about 1 cm test tube were dipped in beaker containing water. Withdraw 10 ml of sample every 30 minutes interval and replace by equal volume of water to maintain the sink condition. Absorbance was taken spectrophotometrically.

RESULT AND DISCUSSION:

Preformulation studies: Isoniazid was a white crystalline powder, freely soluble in water, sparingly soluble in alcohol, very slightly soluble in ether. It was melted at 171°C and their pH was found to be 7.1.

The entire calibration curve (Isoniazid) was found to obey the Beer-Lambert's law within the concentration range of 5-25 µg/ml in respective 0.012M HCl solution. The calculation of drug

content and *in-vitro* drug release were based on these calibration curves.

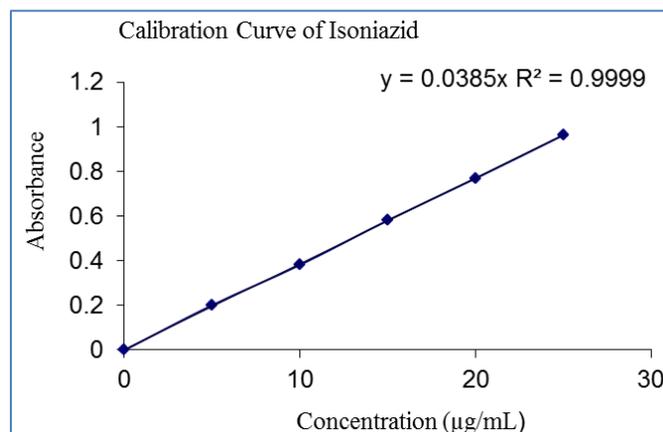


FIGURE 1: STANDARD CALIBRATION CURVE FOR ISONIAZID IN 0.012M HCl

Formulation studies: Isoniazid was reported unstable in aqueous medium and their highly acetylation occurs in liver. Hence enhanced the stability and prevent the acetylation by method of microencapsulation. Microcapsules were prepared by coacervation phase separation method (Temperature change method). Ethyl cellulose was insoluble in cold cyclohexane when temperature increased about 70-75°C it become soluble. Microcapsule prepared by temperature changed method was found to be fine, non-staining, coarse and free flowing characteristics.

Microcapsules of isoniazid were compared with pure drug shows changes in physical properties. Isoniazid, a white colored residue was changed into irregular free flowing behavior after microencapsulation. Particle size was determined with the help of optical microscope (**table 1**).

TABLE 1: PARTICLES SIZE ANALYSIS BY MICROSCOPE

Particle size ranges (µm)	No. of particles (F)	Mid-point (X)	D=X-A A=87.5	D²=D/H H=25	FD'
0-25	26	12.5	-75	-3	-78
25-50	23	37.5	-50	-2	-46
50-75	20	62.5	-25	-1	-20
75-100	15	87.5	0	0	0
100-125	10	112.5	+25	+1	+10
125-150	6	137.5	+50	+2	+12
	ΣF=100				ΣFD' = -122

$$\begin{aligned} \text{Mean} &= A + (\Sigma FD' / \Sigma F \times H) = 87.5 + (-122 / 100 \times 25) \\ &= 87.5 - 30.5 \\ &= 57 \mu\text{m}. \end{aligned}$$

Microcapsules of isoniazid shows enhanced flow properties. Ethyl cellulose formed a film around the drug particles, which suppressed the generation of excess charge and finally improve the flow properties. Angle of repose and bulk density were decreased after microencapsulation. Moisture content of pure drug was decreased after microencapsulation which hint enhancement in stability. Dissolution studies of the drugs and

microcapsules were carried out in pH 7.4 phosphate buffer and graph of % drug released vs. time was plotted. Isoniazid in pure form released 90% about in 50 minutes. After microencapsulation T90% was increased from 50 minutes to about 4 hours (table 2, figure 2).

Permeation test: The microcapsules of Isoniazid drug sustained the release through egg membrane with comparison to pure drug (figure 3).

TABLE 2: EVALUATION OF MICROCAPSULES OF ISONIAZID

Test	Observation	
	Isoniazid	Isoniazid microcapsules
Visual appearance	Fine, white, crystalline.	Fine, white, irregular shape
Size distribution by microscopy		57µm
Bulk Density:		
a) Poured density	0.68gm/mL	0.38 gm/mL
b) Tapped density	0.79gm/mL	0.56gm/mL
c) Carr's index (%)	13%	32%
Flow Property:		
a) Angle of repose	29.98 ⁰	23.56 ⁰
b) Flow rate	0.5gm/sec	0.85gm/sec
c) Coefficient of friction	0.576	0.436
Moisture content	0.58%	0.12%
pH	6.8	5.9

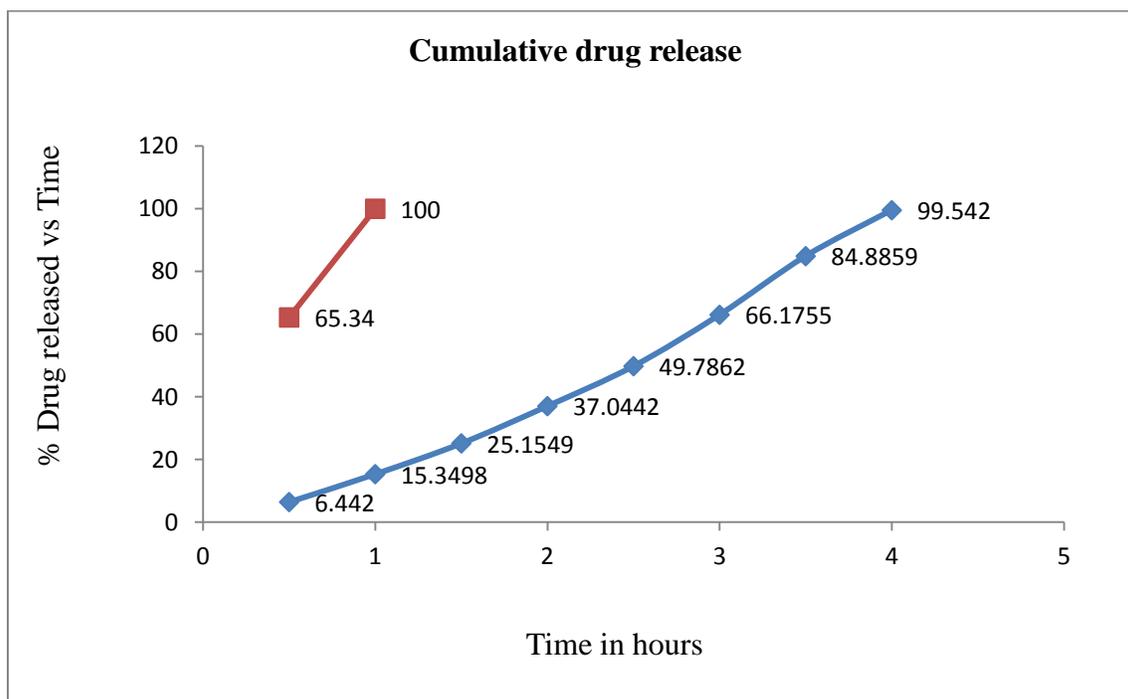


FIGURE 2: DISSOLUTION STUDIES OF PURE DRUG AND MICROCAPSULES OF ISONIAZID

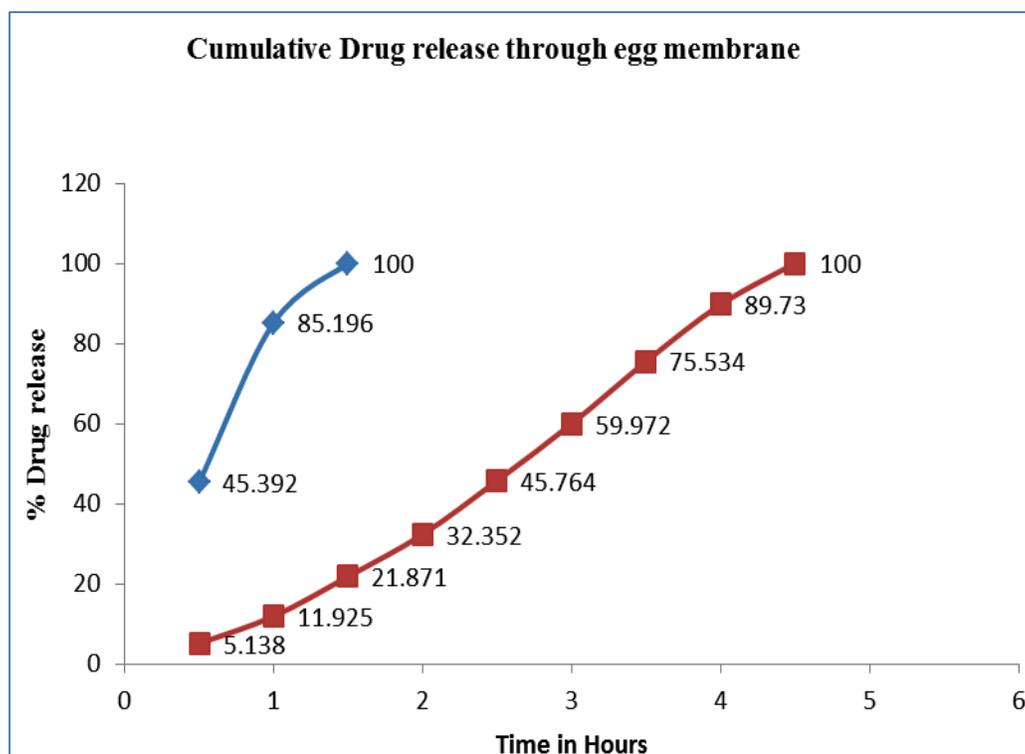


FIGURE 3: PERMEATION TEST STUDIES OF PURE DRUG AND MICROCAPSULES OF ISONIAZID.

So aqueous instability of isoniazid pure drug was minimized by microencapsulation and improve the released of drug means sustained the release of drug.

CONCLUSION: Isoniazid was successfully microencapsulated using ethyl cellulose by phase separation Coacervation induced by temperature change method by using the combination of Isoniazid and ethyl cellulose in the ratio of 1:2 (Drug: polymer). Microencapsulation resulted in significant improvement in physical properties of the drugs especially with respect to flow properties, bulk density and organoleptic properties. Microencapsulation 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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