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RIFAMPICIN AND ISONIAZID MICROCAPSULES FOR TREATMENT OF TUBERCULOSIS

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ABSTRACT

Rifampicin and Isoniazid microcapsules were prepared by phase seperation coacervation method for inclusion in suspension formulations. Ethyl cellulose was used as the coating material for microencapsulation. Developed microcapsules were characterized for visual appearance, photomicrography, sieve analysis, drug content, bulk density, ethyl cellulose content and drug release studies. Microcapsules were found to be irregular in shape, free flowing with wide particle distribution in the range of 178-422 µm with 75 % drug content. Both Rifampicin and Isoniazid microcapsules exhibited prolonged release with first order kinetics. Rifampicin and Isoniazid microcapsule suspension formulation was characterized for pH, viscosity, sedimentation rate and drug content. The developed suspension was found to be uniform with drug content between 99-100%. Stability studies indicated that Rifampicin and Isoniazid suspension formulation exhibited greater stability as compared to pure drug suspensions. Hence, Rifampicin and Isoniazid suspension exhibits a potential to be developed as controlled release paediatric and geriatric formulation.

INTRODUCTION: Tuberculosis is one of the major communicable diseases in developing countries. Since there was a risk of development of drug resistance during treatment, multiple drug therapy of active diseases was established as a means of avoiding drug resistance, allowing shorter duration of therapy and reduced treatment failures.

Rifampicin and Isoniazid were the most popular and conventional drugs for management of tuberculosis [1]. However, Rifampicin and Isoniazid were not used as a single line treatment because of the development of resistance towards this disease. Combined use of Isoniazid and Rifampicin increased the half life of both the drugs and resulted in significant shortening of the period required for successful therapy [2].

Liquid dosage forms with combination of the above drugs would be a preferred option for paediatric and geriatric patients. However, the constraint in developing such formulations was the instability of Rifampicin in aqueous systems [3].

Microencapsulation process employed applying thin coat to small particles of solids or droplets of liquids and dispersions ranging from several tenth of micron to 5000 µm in size [4]. It was a modified form of film coating differing only in the size of particles to be coated and the methods by which this was accomplished [5]. It provided means to convert liquids to solids, separate reactive components and control the release of materials [6].

Microencapsulation had been effectively used in developing sustained release dosage forms and improving stability of drugs. Hence, attempts were made to stabilize Rifampicin in aqueous medium by microencapsulation and formulate it along with Isoniazid microcapsules in suspension formulation.

MATERIALS AND METHODS: Rifampicin and Isoniazid were a kind gift from Lyka labs ltd. Ethyl cellulose and other cellulosic polymers were obtained from Merind Pvt. Ltd. All other reagents and chemicals used in the study were of analytical grade.

Preparation of Rifampicin and Isoniazid Microcapsules by Temperature Change Method: Cyclohexane (solvent) was continuously stirred at 200-400 rpm and maintained at 55^{o}C. Wall material ethyl cellulose was added to the above solution depending on the core: coat ratio. The temperature of the solution was then raised to 70^{o}C for solubilisation of ethyl cellulose in the solvent and maintained over a period of one hour.

Accurately weighed core material (drug) was added to the above solution and the temperature was maintained between 70-75^{o}C for 30 minutes. After this, the system was allowed to cool to 35^{o}C over a period of 4-5 hours. Cooling was further accelerated within a period of 10 minutes upto a temperature of 20-25^{o}C. Drastic cooling was achieved with the aid of ice to a temperature of 5-10^{o}C.

The microcapsules thus formed were then separated by decanting cyclohexane. Filtration was performed using a Buchner funnel at the vacuum pump with Whatman filter paper number 1. They were then rinsed with n-hexane and dried at 45^{o}C for 3-4 hours. The dried microcapsules were stored in well closed containers.

Preparation of Suspensions of pure drugs/ Microcapsules: Sucrose syrup was prepared by dissolving it in 1/3rd of the water required for the suspension. Sodium metabisulphite, sodium citrate, ascorbic acid, sodium edetate, methyl paraben and propyl paraben were dissolved in water. Sodium CMC was triturated in small quantities with the syrup to give a clear uniform base. Dissolved solids along with the syrup were added to sodium CMC base. 0.2 % of Tween 80 was added to the above mixture and triturated lightly to form the suspension vehicle. Microcapsules/pure drugs were then added in small portions and mixed gently with the vehicle and final suspension was obtained by making up the volume.

Characterization of Microcapsules:

Evaluation of Microcapsules:

- **Colour and Visual Appearance:** Pure drug and microcapsules were observed for their overall appearance. Rifampicin microcapsules were specifically checked for staining by holding lightly between two fingers.

- **Photomicrography for Film Thickness** [7]**:** A representative sample of the drug/microcapsules dispersed in glycerin was mounted on a microscopic slide and was observed under a 45 X objective lens. Film thickness of 50 particles from different fields was measured.

- **Particle Size Distribution:**

  - **Microscopy:** Drug /microcapsules dispersed in glycerin were mounted on a microscopic slide and were observed under a 45 X objective lens. Size of 100 particles in 10 different fields was measured.

  - **Sieve Analysis:** 8 gms of microcapsules were weighed and placed on the uppermost sieve of a nest of sieves and were mechanically shaken by vibratory motion. The samples retained on each sieve were weighed and the weight distribution was determined.

- **Bulk Density:**

  - **Aerated Bulk Density (ρ_{ab}):** 8 gm of microcapsules were accurately weighed and carefully poured into a clean, dry measuring cylinder. The volume of microcapsules in the measuring cylinder was noted as V_{ab} and density was determined using the formula:

$$\rho ab = M/ V_{ab}$$

- **Tapped Bulk Density (ρ_{pb}):** Microcapsules were placed on a bulk densitometer which was set for 100 strokes. After being subjected to 100 taps, the volume of the microcapsules in the cylinder was noted as V_{pb} and density was determined using formula:

$$\rho pb = M / V_{pb}$$

- **Flow properties:**

  - **Angle of Repose:** 8 gm of microcapsules were poured through a clean dry funnel clamped on a stand. Microcapsules were collected on a graph paper in the form of a pile by allowing them to fall through the funnel undisturbed. Height and radius of the pile were measured and angle of repose was calculated.

  - **Flow Rate:** 8 gm of microcapsules were accurately weighed and poured through a clean dry funnel clamped on a stand. The time (t) required for the microcapsules to fall through the circular orifice of the stem of the funnel was determined.

Flow rate = Weight of the microcapsules taken/'t' in seconds

  - **Coefficient of Friction:** The tangent of angle of repose was defined as coefficient of interparticulate friction. A μ value of less than one indicates good flow behaviour and μ value of more than one indicates that the material is devoid of flow.

$$\mu = \tan \theta$$

Where μ = coefficient of friction θ = angle of repose

  - **Moisture Content:** One gram of microcapsules were dried at 105°C for one hour. The loss in weight was determined.

Moisture content = (Loss in weight/Original weight) * 100

Drug Content Determination:

- **For Rifampicin and Isoniazid Microcapsules [8]:** Weighed quantities of microcapsules were dissolved in 100 ml of methanol: water (2:3) mixture. This was further diluted to obtain concentrations in the calibration range. The amount of drug present in microcapsules was determined by extrapolating from standard curve.

- **Ethyl Cellulose Content:** 500 mg of the microcapsules were weighed and ethyl cellulose film was dissolved using carbon tetrachloride as the solvent. The dispersion was then filtered and residue was further rinsed with carbon tetrachloride. The filtrate obtained was further dried in oven at 45°C till a constant weight was obtained. In order to account for the drug which may have dissolved in carbon tetrachloride, the residue obtained on drying the filtrate was weighed and dissolved in methanol: water mixture and analyzed spectrophotometrically. The amount of both the drugs dissolved in CCl_4 and this quantity was subtracted form the original weight of the residue to obtain the actual content of ethyl cellulose in microcapsules.

Dissolution studies of Microcapsules: 100-200 mg equivalent to 100 mg of the drug (depending on core: coat ratios) of the microcapsules and 100 mg of the pure drug were weighed. They were individually filled in hard gelatin capsules. Each capsule was placed in USP XXII dissolution apparatus and the studies were performed using 900 ml of pH 7.4 buffer at 100 rpm. Tween 80 was added into each vessel to facilitate wetting of microcapsules. 10 ml of the sample was withdrawn at regular intervals which were further diluted with pH 7.4 buffer and absorbance was measured at 334 nm and 263 nm for Rifampicin and Isoniazid respectively using U.V spectrophotometer.

Evaluation of Suspensions:

- **pH:** pH of both the suspension base as well as suspension of microcapsules was determined using a pH meter MK VI.

- **Viscosity:** The viscosity of the suspension base, suspensions of microcapsules and pure drugs was determined by Brookfield viscometer using LV 3 spindle.

- **Sedimentation Rate:** 10 ml of the suspension was kept in a measuring cylinder and the sedimentation rate was calculated in ml/min.

- **Specific Gravity:** Specific gravity was determined using a specific gravity bottle and was calculated using the formula:

  Specific gravity = C-A/B-A

Where, A = Wt of the bottle; B = Wt of bottle+ water; C = Wt of bottle+ suspension

Drug Content: Since both the drugs were present in combination in the suspension, the amount of each drug retained in suspension was determined by simultaneous equation method. The absorbance of standard solutions of Rifampicin and Isoniazid was measured at both the wavelengths i.e., 263 and 334 nm.

The concentration of Rifampicin in test solution in ug/ml was given by the following equation:

Concentration = $(A_2ax_1 - A_1ax_2 / ax_1ay_2 - ax_2\ ay_1) * 10000$

Similarly, the concentration of Isoniazid in test solution in ug/ml was given by the following equation:

Concentration = $(A_1ay_2 - A_2ay_1 / ax_1ay_2 - ax_2\ ay_1) * 10000$

Stability Studies: Stability studies were carried out for the pure drugs, suspension of pure drugs and suspension of microcapsules. The studies were performed by placing representative samples of each formulation in well closed containers at different temperatures of 12°C, 25°C, 45°C and 60°C as per ICH guidelines. Microcapsules were evaluated for change in appearance, pH and viscosity in case of suspensions and drug content. Log % retained vs. time (days) was plotted for different temperatures. The K value thus obtained was used in Arrhenius plot wherein log K was plotted vs. 1/T. The shelf life (T10%) was thus obtained by extrapolating the K value for room temperature.

RESULTS AND DISCUSSION: Rifampicin was reported to be unstable in aqueous medium. Hence, in order to improve its stability, it was attempted to microencapsulate it using cellulosic polymers. Since Rifampicin and Isoniazid in combination were given as the most effective treatment for tuberculosis, it was decided to prepare a suspension formulation utilizing the microcapsules of individual drugs, especially for paediatric patients. Batch formulas for Rifampicin and Isoniazid microcapsules are as given in **table 1**.

TABLE 1: FORMULAE FOR RIFAMPICIN AND ISONIAZID MICROCAPSULES

Formula code		Core: Coat	Weight of core	Weight of coat	Amount of Cyclohexane (ml)
Rifampicin microcapsules	Isoniazid microcapsules				
A1	C1	3:1	7.5	2.5	130
A2	C2	2:1	6.6	3.3	175
A3	C3	1:1	5.0	5.0	275
A4	C4	1:2	3.3	6.6	350
A5	C5	1:3	2.5	7.5	375

Temperature change method along with Ethyl cellulose was used to induce phase separation coacervation [9]. This method utilized the fact that ethyl cellulose was insoluble in cold Cyclohexane but dissolved completely in Cyclohexane at 70-75°C. Microcapsules obtained by temperature change method were found to be fine, non-staining, granular and free flowing with % yield values between 98-100%.

Evaluation of Rifampicin and Isoniazid microcapsules as compared to pure drugs were given in **table 2 and table 3**. A considerable change in physical properties of the drugs was observed after microencapsulation. Rifampicin which was a brick red coloured staining drug, was found to be non staining and easier to handle after microencapsulation. Isoniazid, an off white coloured powder was transformed into irregular free flowing particles.

Rifampicin film could be easily distinguished by colour difference between the core and coat. In case of Isoniazid, the differentiation between the core and coat could be made by difference in the light transmitted by the two.

TABLE 2: EVALUATION OF RIFAMPICIN MICROCAPSULES

Test	Observations	
	Rifampicin	**Batch A1**
Visual appearance	Fine, brick red coloured, staining, crystalline powder	Irregular shaped, non-staining, free flowing particles
Film thickness	----	5-5.45 µm
Size distribution (By microscopy)	10-50 µm = 27% 50-100 µm = 45% 100-125 µm = 28%	50-100 µm = 3.5% 100-125 µm = 5% 125-150% = 5.5%
Size distribution (By sieve analysis)	< 125µm	<178 µm = 15% 178-251µm = 25% 251-353 µm = 47.5% 353-422 µm = 8% >422 µm = 5%
Bulk density		
a. Aerated (gm/cc)	0.67	0.3
b. Tapped (gm/cc)	0.77	0.32
Flow properties		
a. Angle of repose	46^{o}	24^{o}
b. Flow rate	0.5 gms/sec	2 gms/sec
c. Coefficient of friction	1.04	0.45
Moisture content	0.75%	0.06%
Drug content	97-102%	75%
Ethyl cellulose content	----	21.5%

TABLE 3: EVALUATION OF ISONIAZID MICROCAPSULES

Test	Observations	
	Isoniazid	**Batch C1**
Visual appearance	Fine, off white, free flowing particles	Irregular shaped, free flowing particles
Film thickness	----	5-5.45 µm
Size distribution (By microscopy)	10-50 µm = 37% 50-100 µm = 39% 100-125 µm = 24%	50-100 µm = 0.3% 100-125 µm = 2.5% 125-150% = 5.7%
Size distribution (By sieve analysis)	< 125µm	<178 µm = 10% 178-251µm = 21.5% 251-353 µm = 57% 353-422 µm = 7.5% >422 µm = 4%
Bulk density		
a. Aerated (gm/cc)	0.71	0.45
b. Tapped (gm/cc)	0.80	0.5
Flow properties		
a. Angle of repose	30º	25º
b. Flow rate	1.6 gms/sec	2.67 gms/sec
c. Coefficient of friction	0.595	0.475
Moisture content	0.60%	0.08%
Drug content	98-101%	75%
Ethyl cellulose content	----	26%

For particles size distribution determination, both the drugs were individually sieved through 120 mesh sieve, hence all the drug particles were below 125 µm. Also, the microcapsules obtained were of wide range due to the irregularity of the particles which were originally used. It was also due to the aggregation of particles during microencapsulation that a wide particle size distribution was observed. Lumps were observed to an extent of 0.3-6% of the total yield. Both the drugs exhibited improved flow properties upon micro-encapsulation. This property of microcapsules was found to be maintained even after three months of storage.

Ethyl cellulose formed a film around the particles and prevented the development of excess charges, thereby making the material free flowing. It was also seen that there was a decrease in angle of repose with an increase in proportion of ethyl cellulose in the microcapsules. Bulk density of both the drugs was found to be reduced after microencapsulation. Lower density thus resulted in lighter microcapsules thereby facilitating suspendability.

Moisture content for microcapsules was reduced as compared to pure drugs suggesting improvement in stability. Individual values for drug content were close to the theoretical value indicating reproducibility of the microencapsulation technique. Ethyl cellulose content was found to be in the range equal to theoretical percentage and thus indicates reproducibility of the procedure. Photomicrographs of the pure drugs in comparison with microcapsules exhibited irregular shaped microcapsules (**Figures 1-4**).

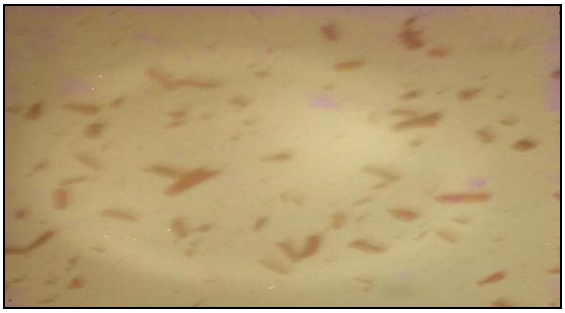

FIG.1 PHOTOMICROGRAPH OF RIFAMPICIN

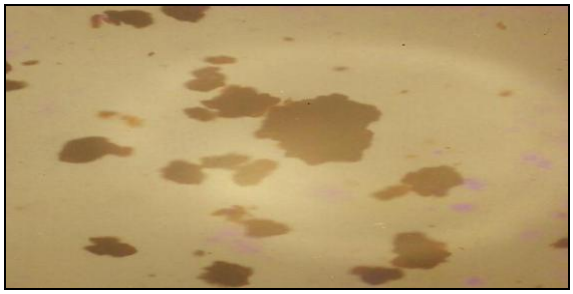

FIG. 2: PHOTOMICROGRAPH OF RIFAMPICIN MICROCAPSULES

FIG. 3: PHOTOMICROGRAPH OF ISONIAZID

FIG. 4: PHOTOMICROGRAPH OF ISONIAZID MICROCAPSULES

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.

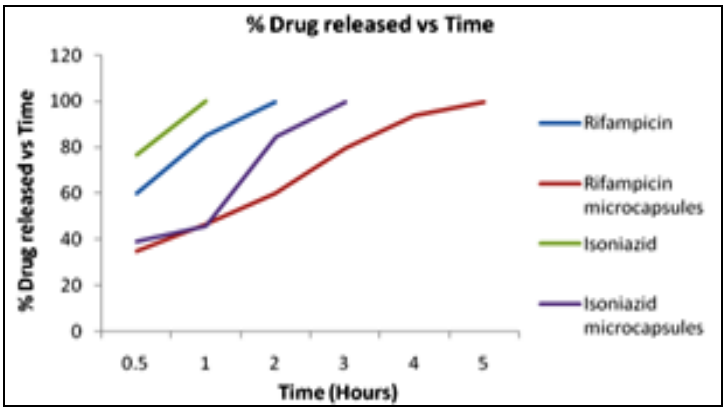

FIGURE 5: DISSOLUTION STUDIES OF PURE DRUGS AND MICROCAPSULES

Rifampicin in pure form was found to release 90% of the drug in 1.16 hours as indicated by figure 5. On microencapsulation, T_{90}% was found to be increased upto 4.79 hours indicating considerable retardation in rate of dissolution. Similarly, for Isoniazid, T_{90}% was increased from 0.82 hours to 3.11 hours for microcapsules as compared to pure drug. Both the drugs were observed to follow a first order release pattern (**table 4**).

TABLE 4: EQUATIONS AND REGRESSION COEFFICIENTS FOR DRUG RELEASE STUDIES

Formulation	Equation	R^2 Value
Rifampicin	Y= 19.93x + 41.75	0.978
Rifampicin microcapsules	Y= 13.84x + 20.68	0.984
Isoniazid	Y= 23.41x+53.15	1
Isoniazid microcapsules	Y= 22.08x + 12.07	0.933

Suspension formulation was prepared and the basic ingredients were chosen based on their compatibility with Rifampicin and Isoniazid. The results of suspension evaluation were as represented in **table 5**.

TABLE 5: EVALUATION OF SUSPENSIONS

Test	Observations	
	Suspension of pure drugs	Suspension of microcapsules
Appearance	Uniform non gritty, brick red coloured suspension	Uniform, brick red coloured suspension
pH	5.65	5.6
Viscosity (cps)	700	1150
Sedimentation rate (ml/hr)	1	1
Ease of redispersibility	Disperses uniformly	Disperses uniformly
Specific gravity (gm/cc)	1.15	1.28
Drug content (%)	Rifampicin: 99.9 Isoniazid: 99.95	Rifampicin: 99.8 Isoniazid: 99.87

Above results indicated that the pure drugs and microcapsules were well dispersed in suspension. Also, the physical appearance on storage showed that the drug suspension possessed a tendency to cake partially as compared to microcapsule suspension. Shelf life of the pure drug suspensions and developed suspension was determined as indicated in **table 6**. It was seen that shelf life of Rifampicin and Isoniazid microcapsules in suspensions was significantly improved as compared to pure drug suspension indicating the ability of ethyl cellulose coating to retard drug degradation in aqueous media.

TABLE 6: ACCELERATED STABILITY TESTING OF SUSPENSIONS

Temperature (^oC)	K (per week)		
	Rifampicin suspension	Isoniazid suspension	Rifampicin & Isoniazid microcapsule suspension
12	$0.31 * 10^{-2}$	$1.09 * 10^{-5}$	9.55*10-4
R.T	$3.24 * 10^{-2}$	$7.24 * 10^{-4}$	5.62*10-4
45	$1.78 * 10^{-2}$	$6.17 * 10^{-4}$	5.49*10-4
60	$1.02 * 10^{-2}$	$4.47 * 10^{-4}$	3.72*10-4
T 10% (Shelf life in Weeks)	3.5	141	150

CONCLUSION: Rifampicin and Isoniazid were successfully microencapsulated using ethyl cellulose by phase separation coacervation induced by temperature change method. Microencapsulation resulted in significant improvement in physicochemical properties of the drugs especially with respect to flow properties, bulk density and organoleptic properties. Microencapsulation also resulted in prolonged release of the drugs. Microcapsule suspension formulation was easily dispersible as compared to suspension of pure drugs.

However, only a marginal increase in Rifampicin stability was observed in aqueous suspension after microencapsulation as compared to Isoniazid. Hence, microencapsulation resulted in improving the stability and prolonging the release of drugs which possessed a potential in formulation of liquid dosage forms for paediatric and geriatric use.

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