Respirable PLGA Microspheres Containing Rifampicin for the Treatment of Tuberculosis: Manufacture and Characterization

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Purpose. Particles with aerodynamic diameters of 1–5 μm deposit in the periphery of the lungs and are phagocytized by alveolar macrophages, the primary site of *Mycobacterium tuberculosis* infection. Aerosols of biodegradable polymeric microspheres containing antitubercular agents may be delivered to the lungs to improve the treatment of tuberculosis.

Methods. Poly(lactide-co-glycolide) (PLGA) microspheres containing rifampicin were prepared using solvent evaporation and spray drying methods. The solvent evaporation process was optimized using factorial experimental design and surface response methodology. The morphology, particle size, drug loading, and dissolution of microspheres was evaluated.

Results. The spray dried rifampicin loaded PLGA microparticles were shriveled, unlike the spherical particles produced by solvent evaporation. Drug loadings of 20% and 30% were achieved for solvent evaporation and spray dried products, respectively. The particles prepared by solvent evaporation and spray drying had 3.45 μm and 2.76 μm median diameters by volume, respectively.

Conclusions. Respirable rifampicin loaded PLGA microspheres were produced by both solvent evaporation and spray drying methods. These particles are being evaluated in an animal model of tuberculosis.

KEY WORDS: tuberculosis; rifampicin; PLGA; biodegradable microspheres; aerosols; controlled release.

INTRODUCTION

Tuberculosis (TB) is a leading cause of infectious lung disease, and considered the foremost cause of death due to a single microorganism. Annually worldwide, of the 8 million people who actively manifest the disease, 3 million die. The new cases that arise every year originate from the pool of asymptomatically infected persons, estimated to include one third of the world's population (1). Tuberculosis has become a significant opportunistic disease among populations with a high incidence of acquired immunodeficiency syndrome (AIDS).

The occurrence of TB is most often due to *Mycobacterium tuberculosis* (MTB) infection, and the lungs are the primary site of infection for the systemic pathogen. Problems created by bacterial infection are linked to their ability to survive and multiply inside the body, especially in the lungs, and to the natural immune response of the infected host. Bacteria reaching the deep lung are phagocytized by alveolar macrophages in the first step of pathogenesis. Inside the macrophage the bacteria will either be destroyed, begin replicating, or remain latent indefinitely. If replication is not prevented, the bacilli multiply and will eventually cause the macrophage to rupture. A cell-mediated response is eventually initiated to combat the infection (2).

Current treatments of tuberculosis are limited by their methods of delivery. The oral bioavailability of rifampicin is 90–95% (3). Drug doses above those currently administered would present the risk of toxic side effects since the antitubercular agents are at their maximum tolerated dose for systemic exposure. Targeting antitubercular drug delivery to the lung may increase local therapeutic effect and reduce systemic exposure. It is proposed that biodegradable aerosol microparticles can be used to target delivery of rifampicin to alveolar macrophages. The particles will deposit in the periphery of the lung due to their aerodynamic size, where they will be ingested by alveolar macrophages, and slow dissolution will occur. The aim is to increase local concentration of drug within the macrophages, the host cell for MTB.

The primary goal of this study was to prepare 1–5 μm geometric diameter poly(lactide-co-glycolide) microspheres loaded with rifampicin using both solvent evaporation and spray drying methods. The solvent evaporation method for the production of microparticles for drug delivery was fully developed in the late 1970's (4). Since then, many studies have been conducted to evaluate the process and to develop an understanding of the effects of individual formulation parameters on the final product. Spray drying has also been used to produce drug loaded polymeric microspheres (5). A process of optimization using factorial experimental design and surface response methodology was applied to the solvent evaporation method used in this study.

MATERIALS AND METHODS

Materials

The antibiotic rifampicin was selected for incorporation into polymeric microspheres based on its antitubercular activity and solubility characteristics. Rifampicin (823.0 g/mole) was purchased from Sigma Chemical (St. Louis, MO) The purity was 95% (HPLC), as determined by the manufacturer. The polyester 75:25 poly(DL-lactide-co-glycolide) (PLGA) was used for the production of microspheres and was purchased from Birmingham Polymers (Birmingham, AL). The MW_w of the polymer was 82,500, as determined by the manufacturer. HPLC grade methylene chloride was obtained from Mallinckrodt Chemical (Paris, KY). The surfactants Pluronic F-68 and Tween 80 were purchased from Sigma Chemical (St. Louis, MO), and Fluka Chemical (Ronkonkoma, NY), respectively. The bulking agent used in the continuous phase, polyvinyl alcohol (PVA), obtained from Polysciences, Inc. (Warrington, PA), was 98% hydrolyzed, and had a MW of 78,000. Glycerol was purchased from Fisher Scientific (Fair Lawn, NJ). All water

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used was Milli-Q grade (Milli-Q System, Millipore, Bedford, MA).

Preparation of Microspheres

Solvent Evaporation

In the typical solvent evaporation procedure, polymer and drug were dissolved in methylene chloride forming the dispersed phase. An aqueous solution containing surfactant and glycerol was used as the continuous phase. The continuous phase was cooled in an ice bath to <10°C and stirred at approximately 5,500 rpm by a high speed dispersator (Premier Mills, Inc., Reading, PA). The polymer/drug solution was injected at 2 ml/min from a glass syringe using a syringe pump (Model 100, KD Scientific, Boston, MA) directly into the continuous phase through a 25.4 cm, 22 gauge stainless-steel needle (Aldrich Chemical, Milwaukee, WI). A high shear rate was responsible for the formation of very small emulsion droplets. Stirring was maintained for 15 min at reduced temperature, then the ice bath was removed and stirring continued for 25–45 min at either ambient temperature or 45°C. Precipitation of solid microparticles followed evaporation of the organic solvent. The microspheres were collected by filtration using filter paper of 0.45 μm pore size (Durapore®, Millipore, Bedford, MA) and washed with 350 ml of water. The product was vacuum dried (Napco® Model 5890, Precision Scientific, Chicago, IL) for a minimum of 8 hr and stored over desiccant at room temperature. The potential for drug and polymer losses during this process necessitated an approach to optimization discussed in the experimental design section below.

Experimental Design

In order to develop an optimal product, a suitable set of processing conditions must be identified. A factorial design approach was applied to the solvent evaporation method of manufacturing rifampicin-PLGA microspheres in order to achieve a maximum drug loading for a product of acceptable size and morphology. It should be noted that maximizing drug loading efficiency was not a priority for this process.

Eight manufacturing variables were selected for evaluation of their effects on the manufacturing process with respect to drug loading and morphology. An initial design of a 2^{8-4} fractional factorial required a total of 16 experiments. A low and high level value were chosen for each variable as shown in Table I. The variable combinations used for each of the experiments are listed in Table II. The factor generators for E, F, G, and H were BCD, ACD, ABC, and ABD, respectively (6). Drug loading (μg drug/mg microspheres) was measured using a high performance liquid chromatography (HPLC) method described later in this text. The percentage of spherical particles observed by scanning electron microscopy (SEM) was used as a quantitative measure of morphology for the experimental products.

Table I. Selected Variables and Level Values for 2^{8-4} Fractional Factorial Design

	Variable	Low level (−)	High level (+)
A	Surfactant concentration (%)	0.04	0.08
B	Solvent volume (mL)	3	5
C	Drug mass (mg)	50	100
D	Buffer (pH)	none	5.2
E	Drug saturation	no	yes
F	Continuous phase volume (mL)	100	150
G	Glycerol (vol%)	70	80
H	Evaporation temperature (°C)	ambient	45

Table II. Run Parameters for 2^{8-4} Fractional Factorial Design

Standard order	Run order	A	B	C	D	E	F	G	H
1	13	−	−	−	−	−	−	−	−
2	1	+	−	−	−	−	+	+	+
3	3	−	+	−	−	+	−	+	+
4	15	+	+	−	−	+	+	−	−
5	6	−	−	+	−	+	+	+	−
6	12	+	−	+	−	+	−	−	+
7	10	−	+	+	−	−	+	−	+
8	8	+	+	+	−	−	−	+	−
9	7	−	−	−	+	+	+	−	+
10	9	+	−	−	+	+	−	+	−
11	11	−	+	−	+	−	+	+	−
12	5	+	+	−	+	−	−	−	+
13	16	−	−	+	+	−	−	+	+
14	4	+	−	+	+	−	+	−	−
15	2	−	+	+	+	+	−	−	−
16	14	+	+	+	+	+	+	+	+

Note: Letters represent variables listed in Table 1.

The final step in the process of optimization was the use of a Box-Behnken design in order to produce a response surface (7). Experimental variations of three parameters were made around a center point totaling 17 experiments. The parameters chosen were solvent volume, drug mass, and polymer mass. The following parameters were held constant: 150 ml continuous phase; 0.08% surfactant; 70% glycerol; buffer pH 5.0; and a 45 min evaporation time at ambient temperature.

Spray Drying

A spray drying technique was also used to produce PLGA microparticles loaded with rifampicin. A 0.5% (w/v) polymer solution was prepared in methylene chloride. An amount of rifampicin was added and dissolved to prepare rifampicin loaded particles with selected drug loading. The solution was spray dried using an instrument (Mini Spray Dryer-Model 190, Büchi, Flawil, Switzerland) with a spray nozzle diameter of 0.5 mm. The process parameters were set to the following: inlet air temperature, 60°C; outlet air temperature, 40°C; aspirator control, 15; 16.7 ml/min liquid feed rate (pump setting, 11); air pressure, 0.45 MPa; air flow rate, 700 NL/h. The liquid feed rate was set to maintain a constant outlet air temperature during the spray drying process. The spray dried product was collected by a cyclone separator. Particles were only recovered from the collection jar and bottom 8 cm portion of the cyclone.

Characterization

Particle Sizing and Morphology

In preparation for scanning electron microscopy (SEM) a small quantity of microspheres (<5 mg) was dispersed in water.

Several drops of the suspension were placed on an aluminum sample stub having previously been coated with adhesive. The samples were evaporated at ambient temperature under vacuum until completely dried, leaving only a thin layer of particles on the stub. All samples were sputter coated with gold-palladium (Polaron 5200, VG Microtech, West Sussex, UK) for 90 seconds (2.2 kV; 20 mA; 150–200 Å) under an argon atmosphere (13.3 Pa). The SEM (Model 6300, JEOL, Peabody, NY) was operated using an acceleration voltage of 10 kV.

Particle size analysis was conducted using SigmaScan™ Pro (Jandel Scientific, San Rafael, CA) image analysis software to assess scanning electron micrographs. A scaling bar was provided with all micrographs and used for calibration of the software's distance measurement feature. The projected area diameter was measured for 200–500 particles from multiple micrograph images and statistical data calculated. A volume median diameter (VMD) was determined for all samples, assuming all particles were spherical and converting the geometric particle size data to a size distribution by volume using the Hatch-Choate equation (8).

Drug Load

Drug loading was determined for microspheres using a modified reverse-phase high performance liquid chromatography (HPLC) method (9). A calibration curve was prepared for rifampicin in a concentration range of 5–150 μg/ml using a solvent medium containing chloroform (1 ml), methanol (9 ml), and dissolved polymer (10 mg). The mobile phase consisted of 50% methanol, 33% phosphate buffer (pH 5.2), and 17% acetonitrile. Analysis was performed on a 50 μL injection at a flow rate of 1.0 ml/min through a μBondapak Nucleosil C_{18} (300 × 3.9 mm) column (Waters, Milford, MA), and the absorbance measured at a wavelength of 337 nm.

A known mass of microspheres was dissolved in 1 ml of chloroform followed by the addition of 9 ml of methanol to precipitate the polymer. The sample was then centrifuged for 10 min at 9,384g and a 1 ml aliquot taken from the supernatant and analyzed by HPLC (Models 710B/510/480, Waters, Milford, MA).

In Vitro Release

Drug release from rifampicin loaded microspheres was carried out using a modified dissolution method (10). The media was a 0.05 M phosphate buffer solution with 200 μg/ml ascorbic acid added as an antioxidant to prevent oxidative degradation. A known mass of microspheres was suspended in tubes of buffer solution at two different pH values, 5.2 and 7.4, to examine the effect of pH on drug release. The pH values were selected to simulate physiologic pH (7.4) and endosomal pH of alveolar macrophages (5.2). Three replicates were used for each pH value. The tubes were placed in a shaker bath at 37°C running at 90 cycles/min. At selected time intervals the tubes were centrifuged and an aliquot of 900 μL taken from the supernatant. A volume of 100 μL of ethanol was added and analyzed by HPLC. A calibration curve was prepared prior to the start of dissolution using a phosphate buffer-ethanol (9:1) media also containing ascorbic acid. The HPLC method used was the same as described above. After the aliquots were removed the entire supernatant was replenished in order to maintain sink conditions. Drug release data was normalized by converting drug concentration in solution to a percentage of the total drug load.

RESULTS

Experimental Design

The purpose of the initial fractional factorial design was to assess the effect of specific manufacturing parameters for the solvent evaporation method. Confounding of variables was necessary to generate the appropriate experiment combinations because this is a fractional factorial design. Specifically, in the design used, all two-factor interactions were confounded with other two-factor interactions. Therefore an effect attributed to a two-factor interaction cannot be isolated without further experimentation. Analysis of the results was focused upon the single variable effects alone. Interactions of three factors or more are generally negligible in comparison to the main effects and so are not considered in the analysis.

SEM was used to image the product collected from each experiment. The particles produced by several of the experiments were not spherical. Consequently, particle sizing could not be performed for the products of these experiments. The remainder of the experiments produced fractions of spherical particles for which the volume median diameter (VMD) of the particles was determined. The particle size did not vary greatly between experiments producing spherical particles with median diameters in the range of 2.4–6.6 μm VMD.

Average effects were calculated for single variables and confounded two-factor interactions. A common method used to analyze effects is the normal-probability plot, as shown in Fig. 1, with respect to drug loading. If all the measured variable

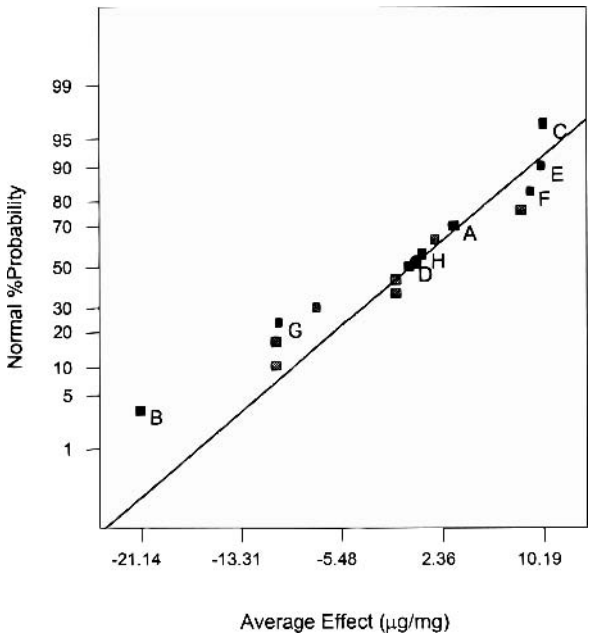

Fig. 1. Normal probability plot of average effects for drug loading response from 2^{8-4} fractional factorial design. Letters represent the variables listed in Table I. The nonlabeled points are confounded two-factor interactions.

effects were insignificant, and due to normal experimental variability, the effects would plot as a straight line having an equal distribution about the zero point. The variable clearly having the greatest effect was solvent volume, with an absolute value more than twice that of the next closest variable. The result indicated that increasing the volume of solvent from 3 ml to 5 ml decreased drug loading. Decreased viscosity of the emulsion droplets can explain the lower drug loading, because the drug more freely diffuses into the continuous phase and decreases the amount eventually entrapped in the polymer matrix.

The same type of analysis was conducted for a second response, morphology. Four of the individual variables had effects greater than 20%, the most significant being drug saturation of the continuous phase. The effect was negative upon the formation of spherical particles. Both, an increased solvent volume and a greater amount of drug used in the dispersed phase, had negative effects on morphology. The use of buffer in the continuous phase had a positive effect upon the formation of spherical particles.

The results of the initial fractional factorial design were considered in developing the Box-Behnken design, so a response surface methodology could be utilized for optimization of the microsphere product. The three factors chosen for evaluation in the design were drug mass, polymer mass, and solvent volume. These factors were selected because an optimal range had not yet been established for them. The values used for the other variables were fixed optimally as determined by prior experimentation.

Examination of the results focused on the responses of drug loading and particle size. The Box-Behnken design is suitable for developing quadratic response surfaces, and constructs a second order polynomial model. The design consists of replicated center points of a cube that defines both the region of interest, and the set of points lying at the midpoints of each edge on the cube. Using Design-Expert® (Stat-Ease Inc., Minneapolis, MN) software, mathematical models were developed from the results of the Box-Behnken design.

The optimization model was constructed by combining the drug load and particle size responses. A desirability function was used to make an overall assessment of the desirability of the combined response variables, drug load and particle size (11). The two responses were equally weighted. The calculated desirability value falls within the interval 0 to 1. A combination of variables resulting in small particle size and high drug load will approach a value of 1. Figure 2 shows the surface response for desirability as a function of solvent volume and drug mass. Simultaneously decreasing solvent volume below 3 ml and increasing drug mass above 100 mg results in higher drug loading and smaller particle size.

The response surfaces modeled predict that both drug load and particle size can be improved further by reducing solvent volume and increasing drug mass beyond the variable range used in the Box-Behnken design. In order to test the model, another experiment was performed using 175 mg of drug, 2 ml of solvent, and 200 mg of polymer. In the presence of a smaller quantity of solvent, 1.75 ml, precipitation of the drug and/or polymer resulted. The microspheres produced had a drug load of 210 μg/mg (21%), a median diameter <5 μm, and were spherical; demonstrating that the model correctly predicted an increase in drug loading and a decrease in particle size.

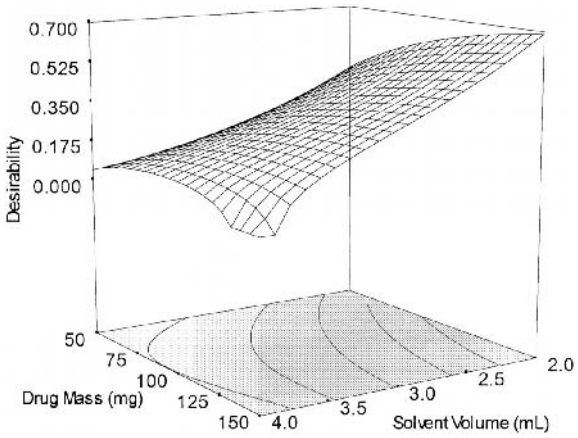

Fig. 2. Surface response of desirability as a function of solvent volume and drug mass. Polymer mass is a constant value of 200 mg.

Characterization

Representative samples of the two methods used to prepare rifampicin loaded microspheres are listed in Table III with their methods of preparation and characterization results. An SEM micrograph of sample EVSR, prepared by solvent evaporation, is shown in Fig. 3, along with a micrograph of a spray dried sample, SP20H. The particles produced by solvent evaporation are shown by SEM micrographs to be generally spherical in shape and having a smooth surface. The spray dried particles are somewhat spherical in that the edges are rounded but the shape is shriveled or "raisin-like." This particle morphology is typical for spray drying and not surprising considering the inlet temperature was above the solvent boiling point and also above the glass transition temperature of the polymer making it less rigid during drying (12). SEM micrographs showed no difference in shape between the spray dried PLGA particles containing significantly different rifampicin loads, 30% (w/w) and 20% (w/w).

In comparing all the samples listed in Table III, particle sizing results show the VMD of the spray dried products to be similar. The size of the non-spray dried products exhibits greater variation compared to the spray dried and the distributions, represented as geometric standard deviation (GSD), are wider.

In both Fig. 4 and Fig. 5, the drug release profiles are shown for selected solvent evaporation samples and spray dried samples respectively, plotted as percent drug release against time. In all cases the release pattern displayed two phases of drug release. A characteristic "burst" effect was observed for all samples to varying degree within the first 24 hours, followed by a period of slower release. The initial rapid release of drug was attributed to release from superficial areas of the microspheres, and may involve both dissolution and diffusion. The second phase of release could be contributed to diffusion of drug through small pores or channels in the polymer matrix.

The pH of the dissolution medium significantly affected drug release, particularly the burst effect. At pH 5.2, the percentage of drug released was lower at the end of the initial phase compared to the higher pH value. This pattern coincides with the dissolution profile of rifampicin alone, suggesting that drug dissolution plays a much greater role in the release kinetics during the initial phase.

Table III. (a) Methods of Preparation and (b) Characterization Results for Selected Samples of Rifampicin-PLGA Microparticles (a)

Sample	Method	Drug/polymer ratio	Polymer solution (% w/v)	Notes
EVTB1	Solvent evaporation	0.50	4.0	Emulsifier PVA/Tween 80
EVTB3	Solvent evaporation	0.50	6.7	Emulsifier Pluronic F-68
EVSR	Solvent evaporation	0.88	10.0	Emulsifier Pluronic F-68
SP30H	Spray drying	0.43	0.5	PLGA MW 82,500
SP20H	Spray drying	0.25	0.5	PLGA MW 82,500
SP30L	Spray drying	0.43	0.5	PLGA MW 54,000

(b)

Sample	Drug load (μg/mg)	Loading efficiency (%)	Yield (%)	VMD (μm)	GSD
EVTB1	43.0	12.9	43.3	4.94	2.00
EVTB3	40.5	12.2	33.3	6.84	2.07
EVSR	207.3	44.4	34.7	3.45	1.77
SP30H	324.0	107.7	41.3	2.76	1.57
SP20H	219.0	109.5	38.8	2.66	1.38
SP30L	328.7	109.3	34.3	2.75	1.41

DISCUSSION

The spray drying method proved to be more effective in producing microspheres with high rifampicin loading compared to solvent evaporation. A drug loading efficiency above 100% was achieved for all spray dried PLGA products. The high loading efficiency is typical of spray drying since the drug cannot partition into an external phase, as is the case with solvent evaporation. A high drug load was achieved for the solvent evaporation product EVSR although the 44% loading efficiency of the preparation process was significantly lower than that of the spray dried product having a similar drug load (SP20H). The method used for the EVSR product included the highest drug polymer ratio and polymer solution concentration compared to all other products.

The average percentage of drug released within 24 hours, 77.1% at pH 7.4, was higher for the spray dried samples than the average of the solvent evaporation samples, 33.6%, at the same pH. This difference can be explained by the difference in preparation methods of the two types. During the manufacturing process for both solvent evaporation and spray drying, droplets are formed containing drug and polymer in solution. The solvent diffuses outward from the center of the droplet toward the surface. The smaller drug molecules tend to move with the solvent. Movement of the polymer is restricted due to intermingling of the long molecular chains, so drug concentrates more at the surface as the droplet dries and precipitates. During the solvent evaporation process much of the drug also diffused into the aqueous phase, whereas during spray drying the drug accumulated near the surface since the droplets are suspended in air. SEM micrographs have shown that the visible surface area

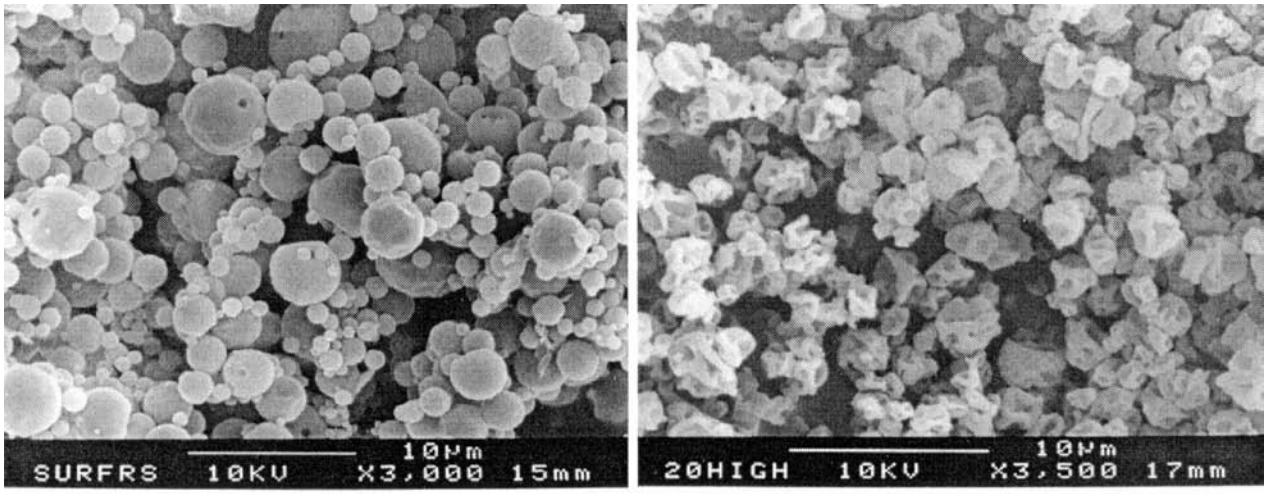

Fig. 3. (a) SEM micrograph of rifampicin-PLGA microspheres, sample EVSR, prepared by solvent evaporation. (b) SEM micrograph of rifampicin-PLGA microparticles, sample SP20H, prepared by spray drying.

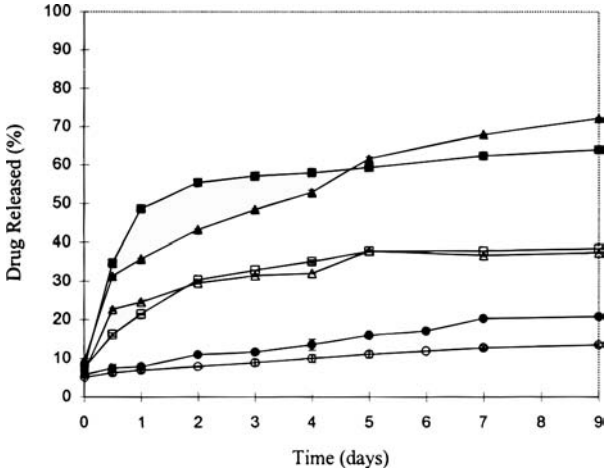

Fig. 4. Drug release in phosphate buffer medium for solvent evaporation samples: EVSR (■), EVTB3 (▲), and EVTB1 (●). Closed symbols are for pH 7.4 and open symbols are for pH 5.2. Points represent the mean of three samples and error bars represent standard error.

of the non-spherical spray dried particles was larger compared to the spherical particles produced by solvent evaporation. The difference in particle shape could also be a contributing factor to the high initial release of the spray dried particles. However, it has been demonstrated that particle porosity is a greater determinant of total surface area than gross morphology (13).

The release profiles of samples SP30H and SP30L were very similar, indicating that the difference in polymer molecular weight did not significantly affect drug release within the time period examined. Had the study been carried out for a longer time period a difference in the release pattern may have occurred due to an earlier onset of matrix erosion for the lower molecular weight polymer (14).

The initial release of drug was higher for the 20% loaded spray dried sample (SP20H) than for the two 30% loaded samples. This result is in disagreement with other studies where a greater initial release was associated with higher drug loading

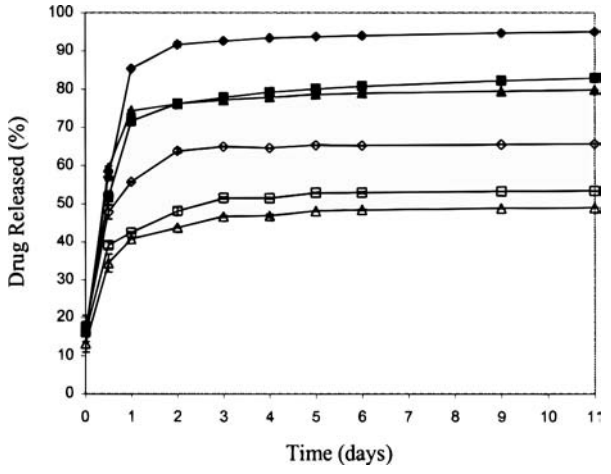

Fig. 5. Drug release in phosphate buffer medium for spray-dried samples: SP20H (♦), SP30L (■), SP30H (▲). Closed symbols are pH 7.4 and open symbols are pH 5.2. Points represent the mean of three samples and error bars represent standard error.

for spray dried particles (15). There may be a limit for the amount of rifampicin that can be partitioned to the outer portion of the microspheres, from which the initial release of drug occurs. If the limit is the same for both the 20% and 30% loaded particles, then the amount of drug released in the initial phase will constitute a higher percentage of the total drug load for the 20% loaded particles. The release rate was slow for all of the spray dried samples during the second phase of release.

The smallest rate of drug release for any sample at pH 7.4 was observed for sample EVTB1. After nine days, less than 20% of the total drug was released. The drug loading of 43 μg/mg was close to that of sample EVTB3. However, close to 70% of the drug was released in the same time period for EVTB3. The particle sizes of EVTB1 and EVTB3 were 4.94 μm and 6.84 μm VMD respectively. A smaller particle size normally causes a faster drug release due to the larger surface area if all other characteristics are the same. The opposite result was observed for these two samples. Additionally, for the preparation of EVTB1, a lower polymer solvent concentration was used, 4.0%, compared with 6.7% for EVTB3. Lower polymer solvent concentration has been shown to increase internal porosity for which a higher release rate would be expected, however, the opposite resulted. There may be a difference in the total surface area between the two samples which was responsible for the difference in drug release, but the cause for the difference in surface area was not particle size or polymer solvent concentration. The only other difference in the method of preparation between the two was the emulsifier type. A combination of PVA and Tween 80 was used for EVTB1 in equal concentrations (0.05%), while Pluronic F-68 was used for EVTB3 at the same concentration. The internal porosity of the microspheres could be affected by the nature of the interface at the surface of the emulsion droplets during manufacture due to the type of emulsifier used (16).

Further investigation is necessary to fully characterize the structure of the microspheres and to explain the different drug release profiles. The scope of this study was limited to investigating the potential for this type of product for the treatment of tuberculosis. Additional experimentation should include surface area measurements. Gas adsorption techniques have been used by other investigators to measure porosity and total surface area of drug loaded polymer microspheres (13,17). It has also been shown that the crystallinity of drug loaded within polymer microspheres (18) and the physical relationship between drug and polymer (19) can affect drug release. Differential scanning calorimetry, X-ray powder diffraction, and infra-red spectroscopy could all be used to further characterize the rifampicin PLGA microspheres.

Rifampicin loaded microspheres have been prepared by others using similar solvent evaporation methods. In two cases, different polymers were used, PLA (10) and a PLA/poly(ethylene glycol) copolymer (20). In a more recent study, PLGA microspheres loaded with rifampicin were produced using different copolymer ratios (21). Methylene chloride was used as a solvent in each study. The particle size and morphology of the microspheres prepared in these studies was similar to ours. However, the drug loading was below 10% in all cases. In no case were the microspheres produced for lung delivery as an aerosol.

CONCLUSIONS

Solvent evaporation and spray drying both proved successful in preparing rifampicin loaded PLGA microspheres with volume median diameters <5 μm. The use of experimental design aided in developing a product with significantly increased drug loading and smaller particle size, without negatively affecting particle shape. In contrast to other studies increasing polymer solvent ratio did not cause larger particles to be formed (20,22). The unique combination of drug and surfactant used for this study may be responsible for the different result. The solvent evaporation method was not efficient with respect to drug loading. The highest efficiency achieved for any formulation was less than 50%.

Spray drying was more effective than solvent evaporation in regard to drug loading. 100% efficiency was observed for all the spray dried products. In addition, smaller particle size distributions resulted from spray drying. Drug release differed between the two types of products in two ways. The burst effect was lower for the solvent evaporation products, and the rate of release generally higher during the secondary phase.

All the characterization results must be considered in evaluating the potential efficacy of the two product types for the proposed treatment. For this reason, no conclusion can be drawn regarding which product will demonstrate the best *in vivo* performance. Based on the particle sizing results alone, aerosol delivery to the periphery of the lungs should be more effective for the spray dried products, either as a dry powder or an aqueous suspension. Therefore, a greater number of drug loaded microparticles are available for uptake by infected macrophages. Better *in vivo* performance for a spray dried product compared to a solvent evaporation product with the same drug loading may result, due to the difference in particle size characteristics. These particles have been evaluated in an animal model of tuberculosis (23); however, investigation of their efficacy is ongoing.

Delivering rifampicin loaded biodegradable microspheres as an aerosol directly to the lungs incorporates several ideas that researchers have been investigating for the potential to improve current TB therapy. Namely: (1) targeting of MTB infected macrophages through the use of microparticles, (2) targeting the primary site of MTB infection, the lungs, and (3) controlled release by incorporating drug within biodegradable particles. Efficacy will be dependent on several factors, including drug release and appropriate aerosol generation.

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