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APPLICATION OF EXPERIMENTAL DESIGN FOR POLY(BUTYLENE SUCCINATE) SYNTHESIS AND OBTAINING RIFAMPICIN-LOADED MICROPARTICLES.

Aplicação do Planejamento Experimental para Síntese do Poli(Succinato de Butileno) e Obtenção de Micropartículas Carregadas com Rifampicina.

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ABSTRACT

Poly(butylene succinate) (PBS), synthesized from the polycondensation of succinic acid with 1,4-butanediol, in two stages, has biocompatibility and biodegradability, but there are still few studies demonstrating the application of this polymer in the biomedical area. The objective of the present work was to use the experimental planning to determine the physicochemical conditions suitable for the synthesis of PBS for nano technology area and to characterize the size and morphology of the microparticle by Low Angle Laser Light Scattering (LALLS) and Scanning Electron Microscopy (SEM) techniques, respectively. To obtain the optimized conditions for synthesis of PBS, the experimental planning was performed by 2k factorial design, which presents two levels of variation (levels +1 and -1) and k experimental factors (X), in which the experimental factors chosen, in the first synthesis step of the polymer were the monomer ratio (X1), temperature (X2) and reaction time (X3); and the experimental

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factors chosen for the second step were the amount of catalyst (X1), temperature (X2) and reaction time (X3). In the first stage, the conditions were: ratio between monomers of 1:1, temperature of 150ºC, time of 5 hours, under constant agitation in an inert nitrogen atmosphere. In the second stage, the conditions were: addition of 3 drops of titanium tetrabutoxide catalyst, temperature of 200ºC, time of 8 hours, under continuous vacuum stirring. Rifampicin is part of the combination of drugs used in the treatment of Tuberculosis. Due to the biocompatibility of PBS, the hydrophobic drug can be gradually released, so it developed a microencapsulated system of rifampicin in PBS by the solvent evaporation method, obtaining an average diameter of 23.3μm with spherical and porous surface structure morphology, however with collapses (ruptures). In conclusion, the experimental planning was essential to reduce analysis time and costs when dealing with PBS synthesis, and to determine the reliability of the results obtained, with synthesis yields of the PBS polymer in the range of 90 to 95%. In future work, a copolymer system with PBS will be developed to nanoencapsulate rifampicin, avoiding the phenomenon of ruptures.

Keywords: Green polymer. Operational Parameters. 2^k Factorial design. Microencapsulation. Controlled drug release. Rifampicin.

RESUMO

O poli(succinato de butileno) (PBS), sintetizado a partir da policondensação do ácido succínico com o 1,4-butanodiol, em duas etapas, possui biocompatibilidade e biodegradabilidade, porém ainda há poucos estudos demonstrando a aplicação deste polímero na área biomédica. O objetivo do presente trabalho foi utilizar o planejamento experimental para determinar as condições físico-químicas propícias para a síntese do PBS para a área nano tecnológica e caracterizar o tamanho e a morfologia da micropartícula pelas técnicas de Espalhamento de luz laser de baixo ângulo (*LALLS*) e de Microscopia eletrônica de varredura (*SEM*), respectivamente. Para obter as condições otimizadas de síntese do PBS foi realizado o planejamento experimental por delineamento fatorial 2^k, que apresenta dois níveis de variação (níveis +1 e -1) e

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k fatores experimentais (X), em que os fatores experimentais escolhidos, na primeira etapa de síntese do polímero, foram a relação entre os monômeros (X1), temperatura (X2) e tempo de reação (X3); e os fatores experimentais escolhidos para a segunda etapa foram a quantidade de catalisador (X1), temperatura (X2) e tempo de reação (X3). A primeira etapa, as condições foram: relação entre os monômeros de 1:1, temperatura de 150ºC, tempo de 5 horas, sob agitação constante em atmosfera inerte de nitrogênio. A segunda etapa, as condições foram: adição de 3 gotas do catalisador tetrabutóxido de titânio, temperatura de 200ºC, tempo de 8 horas, sob agitação contínua a vácuo. A rifampicina faz parte da combinação de fármacos utilizados no tratamento da Tuberculose. Devido a biocompatibilidade do PBS, o fármaco hidrofóbico pode ser gradualmente liberado, portanto desenvolveu um sistema microencapsulado de rifampicina em PBS pelo método de evaporação do solvente, obtendo um diâmetro médio de 23,3μm com morfologia de estrutura superficial esférica e porosa, porém com colapsos (rupturas). Como conclusão, o planejamento experimental foi primordial para a redução de tempo de análise e custos tratando-se da síntese do PBS, além de determinar a confiabilidade dos resultados obtidos, com rendimento de síntese do polímero PBS na faixa de 90 a 95%. Em trabalhos futuros, desenvolver-se-á um sistema copolimérico com o PBS para nanoencapsular a rifampicina, evitando o fenômeno das rupturas.

Palavras-chave: Polímero verde. Parâmetros Operacionais. Delineamento fatorial 2^k. Microencapsulamento. Liberação controlada de fármacos. Rifampicina.

INTRODUCTION

Nowadays, with growing concern for the environment, there is pressure from the world society for the adoption of cleaner production models by industries. One of the focuses of this change is the plastic and rubber industry, responsible for generating several harms to the environment. Within this class of environmentally friendly polymers is poly (butylene succinate), a polyester whose production via the "green" route is economically viable, its mechanical properties are comparable to those of

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commercial polymers (similar to polyethylene and polypropylene) and its degradation process occurs naturally by the environment [1,2].

Few studies have been carried out demonstrating the application of PBS in the biomedical area, however the polymer is biocompatible and biodegradable, due to the susceptibility of its ester groups to hydrolysis, that is, when the polymer degrades by hydrolysis, the hydrophobic drug can be gradually released [3].

The synthesis of this polymer can be divided into two steps: The first is the esterification of succinic acid and 1,4-butanediol to obtain polymers with low molar mass. The second consists of transesterification of the low molar mass polymer, resulting in a higher molar mass and it is necessary to add a catalyst, usually a metal oxide [4].

In order to evaluate the physical parameters used in the polymerization process, such as the amount of these monomers, time and reaction temperature, 2^k factorial design was used in the experimental planning in order to optimize the number of samples. Experimental planning is an essential tool in developing new processes and improving processes in use. Proper planning allows, in addition to improving processes, reducing the variance of results, reducing analysis times and the costs involved [5].

Tuberculosis is an infectious disease caused by bacteria belonging to the Mycobacterium tuberculosis complex commonly transmitted by the inhalation of infectious droplets dispersed in the air by an infected individual through coughing, sneezing or speaking. Treatment for tuberculosis usually involves multiple drug therapy in order to reduce the emergence of resistant strains [6]. Rifampicin is part of the combination of drugs used in the treatment of Tuberculosis, being the only drug insoluble in water, causing problems of bioavailability and the presence of polymorphs, so efforts have been made to seek alternatives for a more effective treatment, including micro and/ or rifampicin-loaded nanoparticles [7].

Microparticles are solid, spherical polymeric systems ranging in size from 1 to 1000 μm. They are subdivided into microcapsules, microspheres and lipidic

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microspheres (SILVA et al., 2003) [8]. In order to develop an innovative product for the treatment of tuberculosis, rifampicin was microencapsulated in PBS and characterized the system by the techniques of Low Angle Laser Light Scattering (LALLS) and Scanning Electron Microscopy (SEM) in order to characterize the microparticle in relation to its size and morphology, respectively. Considering that rifampicin is a hydrophobic drug, the microencapsulation technique by solvent evaporation was used in the preparation of microparticles of the rifampicin and PBS system [9].

MATERIAL AND METHODS

Experimental design for PBS synthesis

The methodology used was the 2^k factorial design, two levels of variation (levels +1 and -1) and *k* experimental factors (X), using the *STATISTICA 5.5* program, in which the chosen experimental factors, in the first step of synthesis of the polymer, were the relationship between monomers (X1), temperature (X2) and reaction time (X3); and the experimental factors chosen for the second step were the amount of catalyst (X1), temperature (X2) and reaction time (X3), Tables 1 and 2.

Table 1: Values of variables at different levels of experimental design for Stage 1 of Polymerization

Factor	Level -1	Level +1
1,4-butanediol: succinic acid, mol (X1)	1:1	1:2
Temperature, ºC (X2)	140	150
Reaction Time, hours (X3)	5	7

Table 2: Values of variables at different levels of experimental design for Stage 2 of Polymerization.

Factor	Level -1	Level +1
Catalyst, drops (X1)	3	4
Temperature, ºC (X2)	180	200
Reaction Time, hours (X3)	8	10

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The dependent variable chosen to obtain the most favorable operating parameters for polymer synthesis was the yield of each polymerization step, since in the literature there is already a range of use in each step, however, in the case of this work, the objective was the synthesis of the polymer for application in the biomedical and pharmaceutical áreas [10].

Synthesis and Characterization of Rifampicin + PBS microparticles

The microencapsulation method by solvent evaporation was carried out, in which rifampicin (a hydrophobic drug) was dissolved in the organic solvent containing PBS. Then, the emulsification of this organic phase (dispersed phase) in an aqueous phase called continuous phase was promoted, followed by the extraction of the solvent from the dispersed phase by evaporation of the solvent, the droplets transforming the dispersed phase into continuous solid particles. Finally, the recovery and drying of the microsphere was carried out to eliminate the residual solvent [11].

The size (number mean diameter) and the size distribution of microspheres were determined by light scattering of the particles suspended in distilled water solutions, using a Mastersizer 2000 (Malvern Instruments). The mean diameter ± standard deviation (S.D.) of six determinations was calculated by applying multimodal analysis. Values reported are the mean diameter ± S.D. for replicated samples. Morphological evaluation of the microspheres was performed using a SEM (Jeol Technics Ltda, JSM-561, Japan). Samples were sputter-coated with a thin gold layer. Surface characteristics were observed at 15 kV and 4-6 A, at a work distance of 10 mm [12].

RESULTS AND DISCUSSION

Experimental design for PBS synthesis

The results for the operational parameters were chosen based on the statistical parameter R^2 (correlation coefficient), Tables 3 and 4, and on the Pareto Chart analysis

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to identify the most significant variables as a function of the prepared polymers, Figures 1 and 2, and thus correlating the influence of variables on Yield with a confidence level of 95% ($p \leq 0.05$) for Stage 1 and Stage 2 of PBS polymerization. The operational parameters obtained as a result of the experimental planning for the first stage were: 1:1 ratio for monomers, reaction time of 5 hours and operational temperature of 150ºC, under constant agitation and inert nitrogen atmosphere. The parameters operational results obtained as a result of the experimental planning for the second stage were: addition of 3 drops of titanium tetrabutoxide catalyst, reaction temperature of 200ºC, under vacuum and continuous stirring for a reaction time of 8 hours [13-14]. These data were used to synthesize the PBS that would perform microencapsulation with rifampicin by the solvent evaporation method.

Table 3: Correlation Coefficient (R^2) and Yield results for each case of factorial planning for Stage 1 of PBS Polymerization.

Case (X1, X2, X3)	R^2	Yield
-1, -1, -1	0.94	90.7
+1, -1, -1	0.90	89.0
-1, -1, +1	0.90	89.1
+1, -1, +1	0.92	89.6
-1, +1, -1	0.98	93.4
+1, +1, -1	0.95	90.4
-1, +1, +1	0.93	89.7
+1, +1, +1	0.91	89.4

Table 4: Results of the Correlation Coefficient (R^2) and Yield for each case of factorial planning for Stage 2 of PBS Polymerization.

Case (X1, X2, X3)	R^2	Yield
-1, -1, -1	0.91	89.0
+1, -1, -1	0.93	90.1
-1, -1, +1	0.95	92.3
+1, -1, +1	0.94	90.7
-1, +1, -1	0.98	94.1
+1, +1, -1	0.94	91.3
-1, +1, +1	0.93	89.7
+1, +1, +1	0.93	89.4

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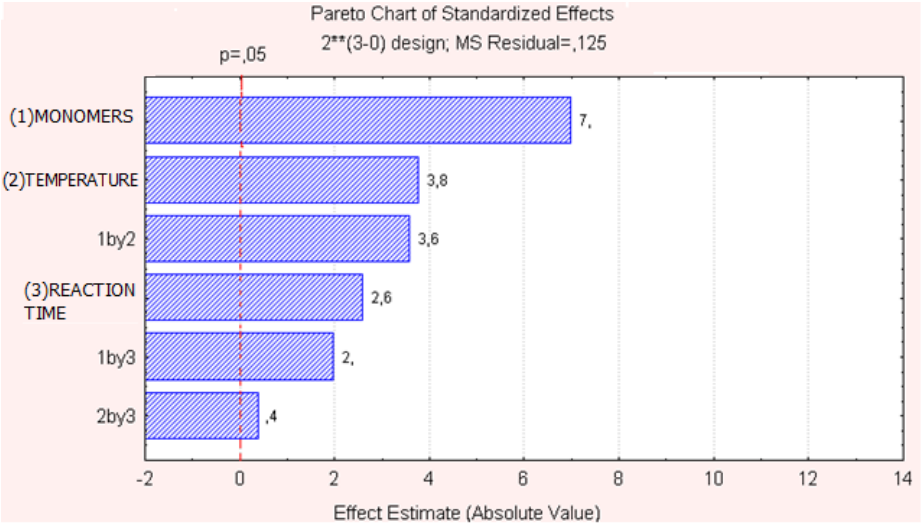

Figure 1: Pareto chart correlating the influence of variables on Yield with a confidence level of 95% ($p \leq 0.05$) for Stage 1 of PBS Polymerization.

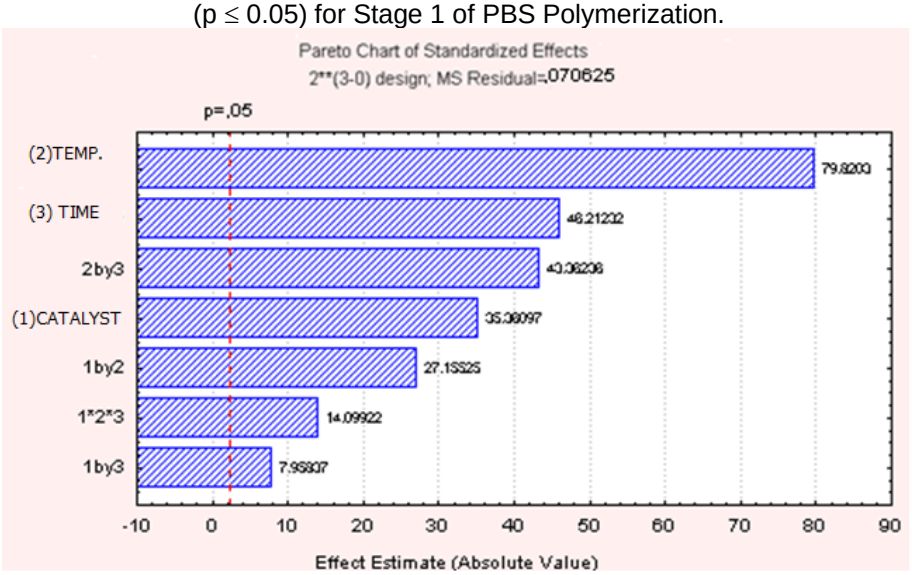

Figure 2: Pareto chart correlating the influence of variables on Yield with a confidence level of 95% ($p \leq 0.05$) for Stage 2 of PBS Polymerization.

Characterization of Rifampicin + PBS microparticles

According to Figure 3, the mean diameter of the microparticles was 23.3 µm, standard deviation of 1.1 µm and volume of 10.2% (data obtained by multimodal analysis of the instrument software). The values indicate that the microencapsulation

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method by solvent evaporation was successful, as micro-sized particles (μm) were obtained.

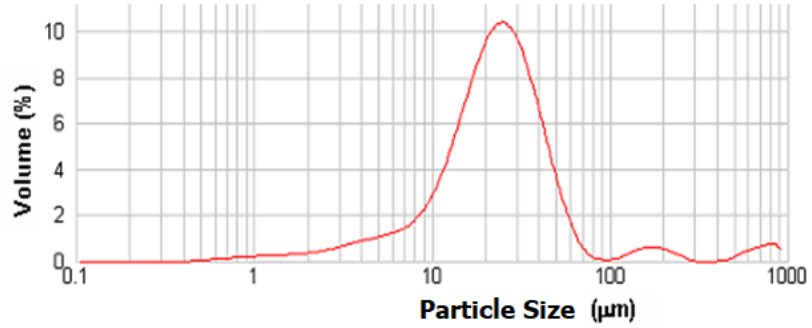
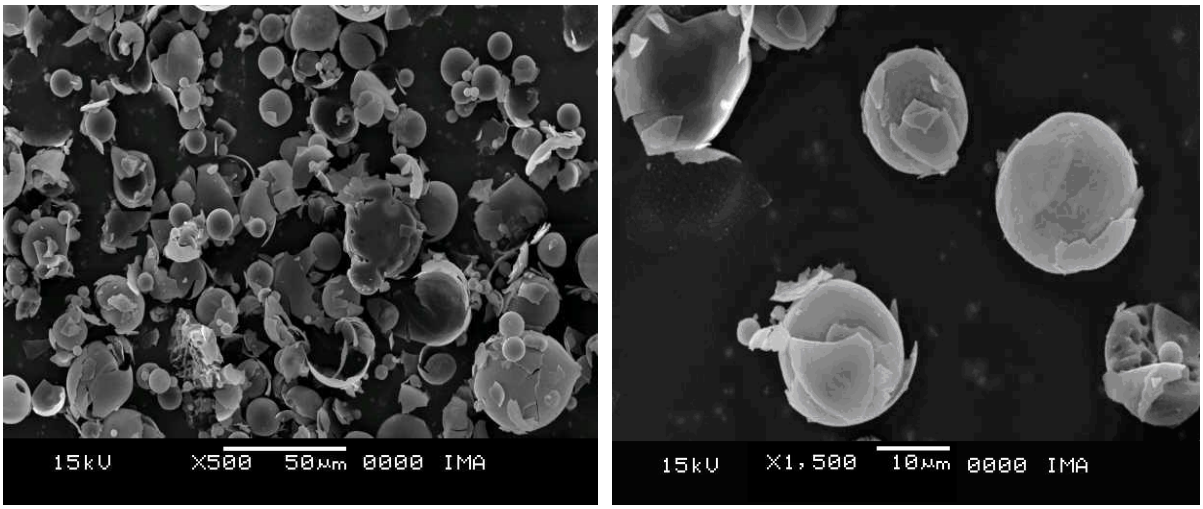


Figure 3: Particle Size Analysis.

Regardless of size, PBS particles loaded with rifampicin formed a spherical surface, but some areas were rough and others smooth, as shown in Figure 4. Similar results were reported by other authors for other polymeric systems. The roughness in some areas can be attributed to the presence of hydrophobic/hydrophilic domains and their separate disposition after the particles drying process [15-16].

Some areas showed a collapsed hydrogel structure, indicating a hollow interior with a porous internal structure, which may indicate that there is a need to develop a polymeric system of PBS with another polymer in order to avoid such a collapsed structure [17-18].



(A) **(B)**

Figure 4: Micrographs with 500x (A) and 1500x (B) magnification.

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CONCLUSIONS

It was observed that the experimental planning was an essential tool in improving the PBS synthesis process with the selection of variables that influenced with a reduced number of bench tests, in addition to determining the reliability of the results obtained, with PBS polymer synthesis yield in the range of 90 to 95%.

The technique for microencapsulation obtained a desirable size for the proposed objective and according to the structure observed in microscopy, a polymeric system of PBS with another polymer must be developed in order to develop nanoparticles of this copolymer with rifampicin to avoid their collapse (ruptures).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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