USE OF EXPERIMENTAL DESIGN AND SIMPLEX OPTIMIZATION ALGORITHM IN THE ENCAPSULATION OF ROASTED COFFEE OIL[1]

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ABSTRACT: Nanoencapsulation is a promising approach to protect the volatile compounds in natural lipid mixtures like roasted coffee oil. In this work, nanocapsules were obtained by the miniemulsification-solvent evaporation technique using poly (L-lactic acid) (PLLA) and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) as encapsulant polymers. The total amount of effectively encapsulated oil was evaluated using a combination of full factorial experimental design and the simplex optimization algorithm and the following independent factors were evaluated: encapsulant polymer, dispersion mechanism, polymer:oil mass ratio and surfactant. The total oil content (oil recovery) was significantly influenced ($p<0.05$) by two-way and three-way interactions confirming that a complex dependence between the factors took place. If PLLA was the encapsulant polymer, then sonication yielded the highest oil recovery. For PHBV as encapsulant, high shear homogenization (Ultraturrax) led to the highest oil recovery. In both cases, polymer:oil ratio and surfactant must be adjusted accordingly.

KEYWORDS: biodegradable nanocapsules, sequential simplex optimization, miniemulsification-solvent evaporation.

USO DE PLANEJAMENTO EXPERIMENTAL E ALGORITMO SIMPLEX DE OTIMIZAÇÃO NA ENCAPSULAÇÃO DE ÓLEO DE CAFÉ TORRADO

RESUMO: A nanoencapsulação é uma alternativa promissora para proteger compostos voláteis em misturas lipídicas complexas como o óleo de café torrado. Nesse trabalho, nanocápsulas foram obtidas pela técnica de miniemulsificação-evaporação do solvente usando poli(L-ácido lático) (PLLA) e poli(hidroxibutirato-co-hidroxivalerato) (PHBV) como polímeros encapsulantes. A quantidade total de óleo efetivamente encapsulada foi avaliada usando uma combinação de planejamento experimental fatorial completo e algoritmo simplex de otimização. Os seguintes fatores independentes foram testados: polímero encapsulante, mecanismo de dispersão, razão polímero:óleo e surfactante. O teor total de óleo (recuperação de óleo) foi influenciado significativamente ($p<0,05$) pelas interações de segunda e terceira ordens confirmando a existência de interações complexas entre os parâmetros. Se PLLA for o polímero encapsulante, então a sonicação forneceu a maior recuperação de óleo. Para o PHBV como encapsulante, o homogeneizador de alto cisalhamento (Ultraturrax0 levou à maior recuperação de óleo. Em ambos os casos, razão polímero:óleo e surfactante devem ser ajustados.

PALAVRAS-CHAVE: nanocápsulas biodegradáveis, otimização sequencial simplex, miniemulsificação-evaporação do solvente.

INTRODUCTION

The oily fraction of roasted coffee is composed mainly by free and esterified fatty acids, diterpenes, sterols and volatiles compounds (Clarke and Vitzthum, 2001). Functional properties of coffee and its by-products were extensively revised as roasted coffee oil is extensively used by the food industry. In addition, there has been some effort to use coffee oil to improve the sensorial properties of instant coffee preparations since aroma is the major factor in purchase decision.
Some approaches were designed to improve the aroma of instant coffee (Patel, 1974). Although they have achieved a relative success, in all cases the volatile compounds were not effectively protected against evaporation. Roasted coffee

oil encapsulation could be a promising alternative to stabilize the flavoring compounds or even to promote their controlled release (Weiss, Takhistov, and McClements, 2006).

The microencapsulation of green or roasted coffee oil was carried out by spray dryer atomization (Chmiel, Liu, Furrer, and Rushmore, 2000; Frascareli, Silva, Tonon, and Hubinger, 2012) and extrusion (Garwood, Mandralis, and Westfall, 1999). However, both processes need high temperatures (90 - 190°C) decreasing the volatile content of the final microcapsules. Coacervation/cross-linking technique (Gaonkar, Nicholson, and Tufts, 1998) was also proposed to roasted coffee oil microencapsulation but the crosslink agent used (glutaraldehyde) is considered toxic. Moreover, it is expected that flavor nanoencapsulation provides better results than microsized capsules, because nanocapsules can promote a faster release from the encapsulated material and present higher degradability, due to its high surface area (Leimann et al. 2013).

The different techniques available to the nanoencapsulation of aromas and other food ingredients were described in the literature (Madene, Jacquot, Scher, and Desobry, 2006) and, in the case of encapsulated food ingredients, all substances must be biocompatible. Biopolymers like poly(lactic acid)(PLA) and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) are of interest because they can be easily shaped as nanoparticles (Weiss et al., 2006). Also, PLA resins are approved by the US Food and Drug Administration (FDA) and European regulatory authorities for all food applications, some surgical applications and as drug releasing systems (Lasprilla et al. 2012). A promising approach of encapsulation is the miniemulsification-solvent evaporation (Leimann, Cardozo, et al., 2013; Loxley and Vincent, 1998; Staff et al., 2013; Urban, Musyanovych, and Landfester, 2009; Y. Zhao, Fickert, Landfester, and Crespy, 2012) since it generally allows an efficient entrapment and the solvent can be readily removed from the final nanoparticles powder. Final particles size was found to be defined by the initial droplets distribution (Musyanovych, Schmitz-Wienke, Mailänder, Walther, and Landfester, 2008). Many operational parameters affect the nanoparticle properties such as homogenizer operating conditions, surfactant type and concentration, encapsulant material and particles composition (Asua, 2002; Boschetto et al., 2013; Fernandes, Marques, Borges, and Botrel, 2014; Staff et al., 2013; X. Zhao, Meng, Liu, and Li, 2014). The interaction between these variables must be well known in order to correctly design the most suitable encapsulation procedure. It is often important to know what combination of polymer/dispersion system favors the encapsulation process. The correct choice of conditions could be difficult to make if complex interactions between the experimental parameters took place and in this case an optimization algorithm could be used. The sequential simplex algorithm is a widely used evolutionary direct search method for solving constrained optimization problems. A simplex is a geometric figure in n dimensions that is the convex hull of (n + 1) vertices and the algorithm iteratively generates a sequence of simplexes to approximate an optimal point (Bona, Borsato, Ferreira, and Paula Herrera, 2000).

In this work roasted coffee oil was nanoencapsulated by the miniemulsification-solvent evaporation technique. Full factorial experimental design and sequential simplex optimization were applied to investigate the oil recovery.

MATERIAL AND METHODS

Lactide (Purac) and tin octanoate (Sigma-Aldrich, 99%) were used in the poly(L-lactic acid) synthesis (PLLA resulting in 3,800 g mol^{-1}). Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV, 8.2 HV mol, Mw 130,300 g mol^{-1}) was kindly supplied by PHB Industrial S.A. Sodium borohydride ($NaBH_4$), dichloromethane, chloroform, hexane, and methanol (Nuclear, P.A.), lecithin (Alfa Aesar) and Tween 80 (Oxiteno) were used in the nanoparticles preparation. Roasted coffee oil was kindly supplied by CIA Iguacú de Café Solúvel.

Nanocapsules were prepared by the miniemulsion/solvent evaporation technique as described elsewhere (Leimann, Biz, et al., 2013). The organic phase was composed by polymer, coffee oil, lecithin and dichloromethane and the water phase was composed by distilled water and Tween 80. In all formulations (Table 1), the organic phase concentration was kept constant at 0.35 $g_{organic\ phase}/g_{total}$. The concentration of the organic phase was kept constant at 0.033 $g_{polymer+oil+lecithin}/g_{organic\ phase}$.

The polymer (PHBV or PLLA) and the hydrophobic surfactant (lecithin) were first dissolved in dichloromethane at room temperature during 10 min under magnetic stirring in a screw capped bottle to avoid solvent evaporation. After that, the roasted coffee oil was added and the stirring was maintained for 5 min. The aqueous phase was prepared dissolving the hydrophilic surfactant (Tween 80) in distilled water under magnetic stirring during 5 min. The organic phase was poured into the aqueous phase and the macroemulsion formed was stirred during 15 min under magnetic stirring. Sonification or high shear homogenization (Ultraturrax) was immediately applied as described below. When the ultrasound device (Fischer Scientific Ultrasonic Dismembrator 120 W, 1/8" tip) was used, the macroemulsion was sonicated in an ice bath for 180 s at 100 % amplitude for 3 minutes in a pulsed regime (30 s sonication, 10 s pause). When the high shear homogenization device (Ultraturrax, T25, IKA) was used, the macroemulsion was emulsified in an ice bath during 10 min at 15,000 RPM. In both cases, the miniemulsion was then transferred to an erlenmeyer flask and dichloromethane was evaporated overnight at 40°C.

A full 2^4 factorial experimental design (16 experiments, Table 2) was applied in order to evaluate the effects of experimental parameters on the roasted coffee oil nanocapsules. The independent factors examined (Table 1) were the type of dispersion system (X_1), polymer (X_2), polymer:oil ratio (X_3) and ratio of surfactant (lecithin:Tween 80, L:T80, X_4). The responses considered were the nanocapsules roasted coffee oil recovery (Y_1) and the nanoparticles average

diameter (Dz, Y_2). Statistical analyses were carried out using the software Statistica 7.1 with a significance level of 95% ($p < 0.05$).

Table 1. Experimental conditions for the factorial design parameters.

Factors	Code	Level	
		(-)	(+)
Type of dispersion system	X_1	High shear homogenization	Sonication
Polymer	X_2	PHBV	PLLA
Polymer:oil ratio	X_3	1:1	2:1
L:T80 (lecithin: Tween80, m:m)	X_4	1:9.4	1:1.1

After the statistical analysis of the experimental data, the model obtained for the oil recovery (Y_1) was maximized to obtain the optimized conditions for each polymer and dispersion system using the sequential simplex algorithm implemented in MATLAB R2008b (Bona et al., 2000). The sequential simplex application is relatively easy, fast, and allows, with a good safety margin, locate the optimal region.

Average diameter of the nanocapsules was determined by Dynamic Light Scattering (Malvern Nanosizer, NanoSeries) by backscattering detection (173°) with samples without dilution. Oil recovery was determined by UV-Vis Spectroscopy (OceanOptics, UV650). The nanocapsules dispersion (1 mL) was dried at 40°C in a circulation oven during 2 hours and then dissolved in dichloromethane (1 mL). After that, methanol was added (1 mL) to precipitate the polymer and the solution was filtered through a 0.45 µm nylon filter. This solution (1 mL) was finally diluted with dichloromethane:methanol(1:1 v/v) in a 10 mL volumetric flask. A blank sample was prepared with the nanoparticles without roasted coffee oil for each sample. The absorbance was determined at 285 nm and the oil concentration was calculated with a previously obtained calibration curve. The oil recovery (R%) was calculated with Equation 1, where C_1 ($mg.mL^{-1}$) is the total oil concentration in the nanoparticles dispersion and C_0 ($mg.mL^{-1}$) is the oil concentration added initially.

$$R\% = 100 \cdot \frac{C_1}{C_0} \quad (1)$$

RESULTS AND DISCUSSION

Experimental Design

The average number molecular weight and polydispersity index of PLLA was 3,800 g mol^{-1} and 1.8, respectively. PHBV presented values of 130,300 g $mol^{-1}$and 0.35, respectively. Table 2 presents the responses obtained for the full factorial design (roasted coffee oil recovery (R%, Y_1) and average diameter (Dz, Y_2)).

Table 2. Formulation used (grams) and results for oil recovery (R%, Y_1) and average diameter (Dz, Y_2).

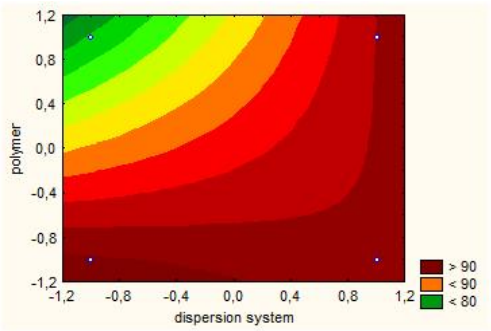
Run	water	polymer	Roasted coffee oil	lecithin:Tween80	dichloromethane	R% (Y_1)	Dz (nm, Y_2)
1	67.320	0.5895	0.5895	0.1146:1.0716	34.680	112.70	263
2	22.440	0.1965	0.1965	0.0382:0.3572	11.560	86.45	173
3	67.320	0.5895	0.5895	0.1146:1.0716	34.680	51.87	180
4	22.440	0.1965	0.1965	0.0382:0.3572	11.560	89.16	178
5	67.320	0.7860	0.3930	0.1146:1.0716	34.680	76.32	257
6	22.440	0.2620	0.1310	0.0382:0.3572	11.560	75.44	208
7	67.320	0.7860	0.3930	0.1146:1.0716	34.680	59.22	260
8	24.440	0.1310	0.2620	0.0382:0.3572	11.560	95.77	263
9	67.320	0.5895	0.5895	0.4701:0.5358	34.680	96.16	213
10	22.440	0.1965	0.1965	0.1567:0.1786	11.560	94.50	271
11	67.320	0.5895	0.5895	0.4701:0.5358	34.680	80.33	357
12	22.440	0.1965	0.1965	0.1567:0.1786	11.560	93.90	201
13	67.320	0.7860	0.3930	0.1146:0.5358	34.680	82.42	314
14	22.440	0.2620	0.1310	0.1567:0.1786	11.560	100.56	152
15	67.320	0.7860	0.3930	0.4701:0.5358	34.680	77.06	227
16	22.440	0.2620	0.1310	0.1567:0.1786	11.560	83.36	219

The statistical analysis (ANOVA) indicated no significant influence ($p>0.05$) of the experimental factors on the average nanoparticles diameter within the experimental range. This means that the average diameter and also the total number of nanoparticles are statistically equal in all experiments, allowing the comparison among oil recoveries. On the other hand, for coffee oil recovery the regression analysis data produced a significant model (Table3).

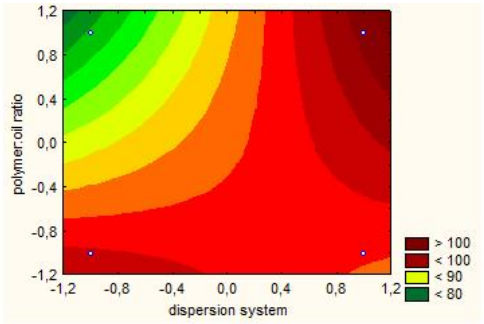
Table 3. Regression model and effects analysis for coffee oil recovery.

	Effect	Standard Error	p	Coefficient
intercept	84,89	0,13	0,00	84,89
X_1	10,76	0,25	0,02	5,38
X_2	-11,36	0,25	0,01	-5,68
X_3	-6,49	0,25	0,03	-3,25
X_4	8,05	0,25	0,02	4,02
X_1X_2	13,42	0,25	0,01	6,71
X_1X_3	5,02	0,25	0,03	2,51
X_1X_4	-0,92	0,25	0,17	-0,46
X_2X_3	7,28	0,25	0,02	3,64
X_2X_4	2,36	0,25	0,07	1,18
X_3X_4	1,87	0,25	0,09	0,93
$X_1X_2X_3$	-6,27	0,25	0,03	-3,14
$X_1X_2X_4$	-11,82	0,25	0,01	-5,91
$X_1X_3X_4$	-1,14	0,25	0,14	-0,57
$X_2X_3X_4$	-8,06	0,25	0,02	-4,03

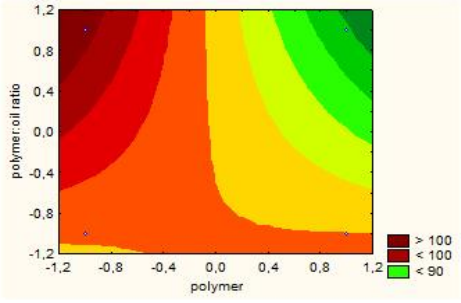
ANOVA analysis indicated an adjusted determination coefficient of 0.9989, indicating a reasonable fit of the regression model. All parameters were significant ($p<0.05$), except the interactions $X_1.X_3.X_4$, $X_3.X_4$ and $X_1.X_4$. However, they were included in the model because they improved the experimental data adjust. The experimental values were reasonably close to the predicted values (data not shown) confirming the validity and adequacy of the predicted model.Figure 1 presents the contour plots for the roasted coffee oil recovery.



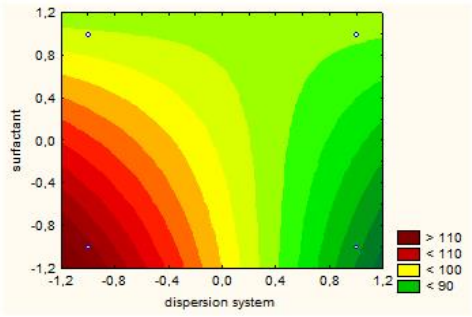
(a) Dispersion system, X_1 and polymer, X_2. Other coded factor levels: X_3 = -1; X_4 = +1.



(b) Dispersion system, X_1 and polymer:oil ratio, X_3. Other coded factor levels: X_2 = -1; X_4 = +1.



(c) Polymer, X_2 and polymer:oil ratio, X_3. Other coded factor levels: X_1 = +1; X_4 = +1



(d) Dispersion system, X_1 and surfactant, X_4. Other coded factor levels: X_2 = -1; X_3 = -1

Figure 1. Contour plot of oil recovery.

The most important linear effects were the encapsulant polymer (PHBV or PLLA, X_2) and the dispersion system (high shear homogenization or sonication, X_1) followed by the surfactant (X_4) and the polymer:oil ratio (X_3). Also, the two-way interactions between them presented statistical significance and therefore must be taken into account in the prediction of the oil recovery in the nanoparticles. Contour plots demonstrated the existence of complex dependence of oil recovery with the experimental variables confirming the importance of the factorial experimental design.

When analyzing these results, it is worth noting that oil recovery is a quantitative indication of the losses during the nanoparticle production either by volatilization or degradation. The experimental conditions that led to high oil recovery values were the use of PLLA combined with sonication as well as use of PHBV combined with high shear

homogenization (Ultraturrax), meaning that in both cases the oil loss was less pronounced. The dispersion time required by the Ultraturrax homogenization was higher (10 minutes) than by ultrasound (3 minutes), since the ultrasound is known to deliver energy more efficiently (Asua, 2002). This means that the Ultraturrax system was more susceptible to oil evaporation, which led to lower oil recovery when PLLA was used. However, oil volatilization was avoided when PHVB was the encapsulant. This could be due to the high viscosity of the PHBV-dichloromethane solution when compared to the PLLA-dichloromethane solution, which led to a more efficient oil protection against volatilization during dispersion. The different behavior for each dispersion system can be attributed to the interaction between the polymer type (X_2) and the other parameters.The same effect was observed in the encapsulation of bovine serum albumin in PLLA and PHBV microparticles by the emulsification-solvent evaporation technique (Conway, Eyles, and Alpar, 1997). The authors correlate the PLLA microstructure with the lower encapsulation efficiency since the hydroxyvalerate content of PHBV has a plasticizing effect reducing its crystallinity.

One can note that the increase in the polymer:oil ratio (X_3) led to a decrease in the oil recovery. This was more pronounced when the Ultraturraxor PHBV were used. This is in agreement with the fact that oil was lost by volatilization during the homogenization step. When more polymer is present, the viscosity of the oil/polymer/solvent increases and the heat generated by the homogenizer could not be efficiently transferred to the water phase. More oil was lost by volatilization when Ultraturrax was used, because the required homogenization time was higher when compared to the sonication time.

The lower surfactant ratio level (X_4) presented a larger amount of the water-soluble Tween 80 which can stabilize the oil outside the nanocapsules through micellization. During the solvent evaporation step, oil outside the polymer matrix can volatilize faster leading to a reduction in the oil recovery. This effect is known to occur in the encapsulation of solid drugs resulting in lower encapsulation efficiency (Eltayeb et al., 2013).

It is often important to know the optimal parameters for a given polymer or dispersion system when designing a production procedure to obtain polymeric nanoparticles. This information can be obtained using the sequential simplex algorithm as presented in Table 4.

Table 4: Optimal conditions obtained via sequential simplex optimization for the coffee oil recovery.

Dispersion System/Polymer[a]	**Coffee oil recovery (%)**		**Polymer:oil ratio**	**Surfactant**
	Predicted[b]	**Observed**		
UT / PHBV	112.6 ± 0.5	112.7	1:1	1:9.4
US / PHBV	100.7 ± 0.5	100.6	2:1	1:1.1
US / PLLA	94.0 ± 0.5	93.8	1:1	1:9.4
UT / PLLA	80.2 ± 0.5	80.3	1:1	1:9.4

a) UT: ultraturrax; US: ultrasound. b) Predicted by the regression model (Table 6).

When PHBV was used as encapsulant, the highest oil recovery (112.6 ± 0.5 %) was achieved with Ultraturrax as the dispersion system. On the other hand, if PLLA is the encapsulant of choice, the use of ultrasound maximized the oil recovery (94.0 ± 0.5%). It is worth noting that polymer:oil ratio and surfactant ratio must be adjusted in each case.

CONCLUSION

Full factorial experimental design evaluated the influence of the encapsulant polymer (PLLA or PHBV), dispersion system (high shear homogenization or sonication), surfactant concentration (lecithin and Tween 80) and polymer:oil ratio on the encapsulation of roasted coffee oil in biocompatible nanoparticles. All linear effects and some interaction effects were significant ($p<0.05$). Sequential simplex optimization demonstrated that the highest oil recovery was obtained combining the encapsulant PLLA with sonication as well as the use of PHBV combined with high shear homogenization (Ultraturrax homogenizer). In general, oil recovery was favored by high polymer:oil ratio due to a viscosity effect. Oil recovery decreased when more water-soluble surfactant was used due to the stabilization of free oil outside the nanoparticles.

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