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Preparation of vancomycin microparticles: Importance of preparation parameters

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Abstract

The aim of the present work was to prepare microparticles containing vancomycin for intraocular injection. The primary objective was to guarantee continuous release and keep an intracameral drug concentration above the minimal inhibitory limit for at least 24 h, needed in endophthalmitis prophylaxis after cataract surgery. Poly(lactide-co-glycolide) microparticles were prepared using the double emulsion (water-in-oil-in-water) solvent extraction/evaporation method. The influence of preparation parameters on the final microparticles properties was explored in an attempt to control particle sizes, stability, encapsulation rate and vancomycin release profile. Satisfying release profile and stability were obtained, independently of the process. Sizes and encapsulation rate were controlled using an experimental design. Final obtained properties demonstrated that the fabricated particles are suitable for the prophylactic intraocular use in cataract surgery. Further *in vitro* and *in vivo* experiments will be conducted to assess efficiency of the entrapped antibacterial and then validate its potential usefulness in prophylaxis.

Keywords: Encapsulation; Endophthalmitis; Vancomycin; Experimental design

1. Introduction

Endophthalmitis is a severe inflammatory process involving both the anterior and posterior segments of the eye; it may result in permanent eye damage and loss of sight. It may be endogenous, and may also occur in postoperative and post-traumatic situations. Postoperative endophthalmitis remains one of the most serious complications after cataract surgery with intraocular lens (IOL) implantation. The occurrence of postoperative endophthalmitis is low and estimated to lay between 0.07 and 0.32% (Kodjikian et al., 2005). However, intraocular contamination appears to be relatively common after uneventful cataract surgery. The rate of culture positivity in anterior chamber aspirates ranges from 0 to 46% (Kodjikian et al., 2005). It has been shown that bacteria routinely enter the anterior chamber during cataract extraction. They may be carried into the eye by irrigation or may adhere to the IOL as it is inserted through the incision.

Vancomycin, a peptide drug, is showing a high antibacterial activity against *Staphylococcus aureus* and other *Staphylococcus* species (Cheung and DiPiro, 1986), reported to be responsible of about 70% of postoperative endophthalmitis (Han et al., 1996). Since this drug is poorly absorbed from the gastrointestinal tract, intravenous administration has been tried. But this route has been found inadequate to achieve therapeutic levels of vancomycin concentration in the aqueous humor (Souli et al., 2001). Furthermore, systemic administration of vancomycin can be associated with severe adverse effects (Reynolds, 1996). A current practice consisting in placing filtered vancomycin (20 μg/mL) into the infusion bottle during cataract surgery (Gills, 1991) is highly controversial (Ferro et al., 1997; Feys et al., 1997; Gordon, 2001). It is nowadays well known that intraocular injection of vancomycin used during cataract surgery fails to maintain an efficient level of the antibacterial over a sufficient period of time, estimated to at least 11 h (Kodjikian et al., 2005). Hence, alternative formulations are needed to extend the time over which vancomycin intracameral level remains high enough and therefore improve the ocular performance of this appropriate antibiotic.

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Microparticulate polymeric delivery systems (microspheres, microcapsules, liposomes, etc.) have been investigated as a possible approach to increase the ocular drug availability (Wiechens et al., 1998; Yeh et al., 2001). In the present investigation, we have developed for the first time a vancomycin based biodegradable poly(lactide-co-glycolide) (PLGA) microparticles for intraocular route, with the aim of allowing an extended release of the antibiotic and an improvement of its intraocular half-life, and hence of its prophylactic efficiency in cataract surgery.

PLGA is a biodegradable, bioresorbable polymer, widely used for drug formulations and medical purposes since it is nontoxic and well tolerated by the human body. Its *in vivo* enzymatic hydrolysis leads mainly to water and carbon dioxide (Yasukawa et al., 2004).

Loading of vancomycin, a hydrophilic antibiotic, into PLGA microparticles can be problematic owing to its high hydrophilicity. The most used technique to encapsulate hydrophilic molecules is the double (water-in-oil-in-water, w/o/w) emulsification method, followed by solvent extraction/evaporation (Vranckx et al., 1996). The solubility and concentration of drug, the type of polymer and organic solvent, the type and concentration of emulsifier, the solution viscosity and temperature are all important factors (Liu and Deng, 2002; Bezemer et al., 2000; Yang et al., 2000a,b), along with the process parameters (Changhong et al., 1994), which determine the final properties of the particles.

We did not find any work in literature that focused on the importance of particle sizes in the intraocular route. The aim of this study was then to fabricate vancomycin microparticles suitable for this route, by using design of experiment to adjust the process parameters for optimal particle sizes. The design of experiment was also used to optimize antibiotic release profile for endophthalmitis prophylaxis. Stability (through the zeta potential) and encapsulation rate were also studied, and the final fabricated particles were characterized in terms of shape, size distribution and stability.

2. Materials and methods

2.1. Materials

Vancomycin hydrochloride was purchased from Merck Génériques (Lyon, France); poly(D,L-lactide-co-glycolide) (PLGA, 50–50%, inherent viscosity 0.15–0.25 dL/g; MW 12,000) was purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany); polyvinyl alcohol (PVA, Mowiol 4–88) was purchased from Seppic (Castres, France); acetonitrile (Ultragradient HPLC grade) was purchased from Mallinckrodt Baker Inc. (Deventer, The Netherlands); methylene chloride (analytical grade) was purchased from Carlo Erba Reagenti (Milano, Italy); phosphoric acid (analytical grade) was purchased from Chimie-Plus Laboratoires (Denicé, France); isopropyl alcohol and potassium dihydrogen phosphate (analytical grade, RP Normapur) were purchased from Merck-Prolabo (Lyon, France); balanced salt solution (BSS) was purchased from Alcon (Nanterre, France); 0.22 μm cellulose acetate filters (Cameo) and 5 mL polypropylene syringes were purchased from Fisher Bioblock Scientific (Lyon, France).

2.2. Preparation of the microparticles

Vancomycin loaded PLGA microparticles were prepared by a modification of the w/o/w solvent extraction/evaporation procedure described by Le Ray et al. (2003). Briefly, vancomycin hydrochloride of 100 mg was dissolved in 1 mL of water to form the internal phase. PLGA of 100 mg was dissolved in 1 mL of methylene chloride to form the intermediate phase. The internal phase was introduced progressively in the intermediate phase and sonicated at 40% amplitude for 1 min (Vibracell®, Bioblock Scientific, France), to form the primary emulsion. This emulsion was introduced dropwise into 10 mL of a 1% PVA solution under high speed mixing (Ultraturrax®, T25 Basic, IKA Werke GmbH, Germany) to form the final double emulsion. Finally, 100 mL of a 5% isopropyl alcohol solution were added and the mixture was left for 1 h under an airflow and mild mixing (75 rpm) at 35 °C.

The microparticles suspension was then centrifuged at 50,000 rpm for 30 min (Optima Co-Max-E Ultracentrifuge, Beckman Coulter Particle Characterization, Miami, FL), and washed twice with distilled water to eliminate adsorbed PVA. The collected particles were freeze-dried and stored at 4 °C for further testing.

2.3. Particle size analysis

The distribution of sizes of the microparticles was determined using laser diffraction technique with a Coulter® apparatus, model LS 230 (Beckman Coulter Particle Characterization, Miami, FL). We considered the percentage of particles with size between 1 and 12 μm (that we called "intraocular suitable particles" or ISP) for the experimental design part. For stability study, size measurements were performed on particles suspended in BSS immediately after their fabrication, and after 2 weeks under magnetic stirring at 100 rpm. In every case, the size distribution was calculated from three measurements made on the same sample. BSS is an intraocular irrigating solution with chemical composition similar to aqueous humor. It is a sterile physiological, lint-free solution containing essential ions for normal cell metabolism. It is isotonic to the tissues of the eye.

2.4. Zeta potential measurement

Zeta (ζ) potential measurements of diluted samples were made with a ZetaSizer® 3000 (Malvern Instruments Ltd., Malvern, UK). Zeta potential values obtained from ZetaSizer were average values from ten measurements made on the same sample. Initial measurements on several samples of the same kind showed that this number is sufficient to give a representative average value. Measurements were taken after the manufacturing process, and repeated after 2 weeks under magnetic stirring in BSS at 100 rpm.

2.5. Particles drug loading

Preparation yields of microparticles varied between 71 and 92% depending on process parameters. We preferred then to calculate the drug loading efficiency by taking into account entrapped and total vancomycin used in the experiment. Then, the suspension of microparticles was centrifuged at 50,000 rpm for 30 min, and the collected microparticles were washed twice with distilled water. The supernatant was used to determine the non encapsulated drug fraction whereas the microparticles were suspended in BSS solution, and destructed using an ultrasound bath at 47 KHz (Branson B-2200 E3, Bransonic Ultrasonic Co., Danbury, CT) for 2 h. This period of time is considered sufficient for total vancomycin liberation according to previous validated works in our laboratory. The resulting suspension was centrifuged and vancomycin concentration was determined by means of HPLC. Each determination was carried out in triplicate. Total vancomycin used was considered as the summon of encapsulated and non encapsulated drug. Drug loading (DL) was calculated as

$$\text{DL}\ (\%) = \frac{\text{entrapped fraction}}{\text{total drug used}} \times 100$$

2.6. Vancomycin in vitro release

Microparticles (30 mg) were suspended in BSS (30 mL to be in SINK conditions) and left under magnetic stirring 100 rpm). They were incubated at 32 °C for 24 h. At appropriate intervals, aliquots (1.5 mL) were recovered and filtrated through 0.22 μm filters to determine the released quantity of antibiotic.

2.7. HPLC measurement

The HPLC unit (Thermosystems, inc. Lombard, IL) consisted in a set of a Spectra System P1000XR pump, a Spectra System AS 300 autosampler and a Spectra System UV 6000LP diode array detector. The data is recorded and analyzed with the Chromquest® PC software over the Spectra System SN4000 unit. Chromatographic separations were performed at 25 °C. HPLC method was a modified method of Farin et al., 1998. Twenty microliters of samples or calibration standards were injected directly into the column and were eluted under isocratic conditions through a Lichrocart® RP-18e column, 125 mm × 4 mm; 5 μm particles with a C18 guard column (Merck, France). The mobile phase was composed of 5 mM potassium dihydrogen phosphate buffer (pH 2.8 adjusted with phosphoric acid)–acetonitrile (97:3, v/v). It was filtered through a 0.45 μm pore size cellulose membrane filter, and degassed with helium flow before use. The total run time was 15 min at a flow rate of 1.5 mL min^{-1}. Each determination was carried out in triplicate.

2.8. Experimental design

The process leading to vancomycin based PLGA particles was optimized using a factorial design of experiment. We focused on the Ultraturrax mixing parameters: time (Tm) and speed (Spd).

Table 1
Correspondence between orthogonal and real values

Level	Values	
	Time, Tm (min)	Speed 2, Spd (rpm)
−1	0.5	8000
−0.813	Not used	9500
−0.313	Not used	13500
0	2.75	Not used
+0.563	Not used	20500
+1	5	24000

Temperature is a critical parameter that can affect microparticles, because of the low boiling point of methylene chloride (40 °C). To avoid excessive heat, we did not exceed a mixing speed of 24,000 rpm a duration of 5 min. We also used an ice bath to keep operating temperature constant during mixing time.

Due to the limitation of the used mixing device, speed variation was limited to five values (8000, 9500, 13,500, 20,500 and 24,000). Time was left as a free variable between 0.5 and 5 min.

Due to the complex interaction between the different factors, the aid of appropriate computer software was needed. Table 1 shows the correspondence between the orthogonal and the real values for the variables involved. They have been distributed at random in experimental blocks and a subset of 11 possible setting combinations was selected (see Table 2) using D-optimal (Johnson and Nachtsheim, 1983) design with Modde (Version 6.0 Umetrics, Sweden). The objective was to identify optimal process parameters leading to particles with sizes and release profile suitable for intraocular route. Good stability and encapsulation rate were also targeted.

Table 2
Factorial design generated combinations (in orthogonal values)

Formula	Parameter	
	Tm	Spd
1	−1	−1
2*	+1	−1
3*	+1	−1
4	0	−0.813
5	−1	−0.313
6	+1	−0.313
7	0	+0.563
8	−1	+0.563
9*	+1	+1
10	−1	+1
11*	+1	+1
12**	0	+1
13**	0	+1
14**	0	+1
15**	0	+1

Asterisk (*) and double asterisk (**) denotes combinations carried more than once for the reproducibility study.

2.9. Scanning electron microscopy

Particles were left for 8 weeks in BSS solution under magnetic stirring (100 rpm) and at 32 °C, to be as close as possible to intraocular conditions (Laroche et al., 1996).

Images were taken at days 1, 14 and 56: particle suspensions were deposited on a metallic probe then placed in liquid nitrogen during 10 min and evaporated under vacuum. Particles were then metallized with gold/palladium with a cathodic pulverizer Technics Hummer II (6 V and 10 mA). The surface morphology of the microparticles was then examined using an FEG Hitachi S 800 SEM at an accelerating voltage of 15 kV.

3. Results and discussion

3.1. Experimental design

With the exception of few studies (Barichello et al., 1999; Le Ray et al., 2003; Gavini et al., 2004), the method used to encapsulate vancomycin is the double emulsion solvent extraction/evaporation method. Formulations are similar in most previous works, but process parameters vary and are believed to influence the particle sizes (Le Ray et al., 2003). We did not find in literature any design of experiment based study of the influence of preparation conditions on particle sizes. So we chose the particle formulation according to research literature and then we used a design of experiment to fix the most appropriate parameters leading to suitable sizes, stability, drug release, and a maximum encapsulation rate.

Experimental design consisted in generating 11 different combinations of process parameters. The generated combinations presented in Table 2 were performed and results (particle sizes and encapsulation rates) were introduced in the model (Table 3).

The four responses (particle sizes, encapsulation rate, vancomycin released at 24 h and zeta potential) were calculated and applied as regressors in a PLS correlation (Wold et al., 1984), using the starting settings as descriptor matrix. The confidence level was 0.95.

The D-optimal design returned a second-degree polynomial correlation between process parameters each response as shown:

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_{ii}^2 + \sum_{i<j}^{k} \sum_{j=1}^{k} b_{ij} x_i x_j$$

Y is the response, x the variable, and b is the regression coefficient. b_i describes the linear quantitative effect of the variable in this model. b_{ij} measures the interaction effect between the variables, and the square term b_{ii} describes non linear effect on the response.

The summary of fit (Fig. 1) showed that our model is acceptable for particle sizes (or ISP) and encapsulation rate, with high model accuracy (R^2) and predictability (Q^2): 0.97 and 0.95 for size and 0.78 and 0.50 for encapsulation rate, respectively. Reproducibility and validity are both higher than 0.60.

Regarding the release at 24 h, the model suffered a very poor reproducibility (0.35). This might be due to uncontrolled process or release parameters such as temperature, luminosity, stirring speed, ... Q^2 was also low showing that our model is not suitable in predicting drug release, based on process parameters.

Finally, zeta potential model suffered also low Q^2 (and then low predictability) and a negative model validity. Negative model validity meant that the model prediction error is larger than the experimental error. This model was thus not usable.

Correlation coefficient for the four studied responses is shown in Fig. 2. As expected, a good correlation between predicted and experimental response was observed for particle sizes and encapsulation rate, and a low correlation for release profile and zeta potential.

Therefore, the zeta potential and the release profile data were discarded in the final optimization analysis. Only particle sizes and encapsulation rate data were retained. Model polynomial

Table 3
Experimental results of the factorial design formulae

Formula	ISP[a] (%)	Encapsulation rate (%)	Release at 24 h[b] (%)	Zeta potential
1	19.11	77.32	92.95	−8.4
2	22.03	89.28	91.33	−6.7
3	35.33	88.54	90.25	−6.9
4	43.47	80.05	92.11	−6.7
5	30.02	90.35	90.02	−7.3
6	52.88	97.62	89.55	−8.35
7	72.4	92.23	90.37	−9.65
8	71.22	92.71	92.22	−6.65
9	70.13	96.33	89.84	−8.5
10	77.95	90.3	91.53	−10.9
11	71.88	95.99	89.22	−10.5
12	100	93.22	91.76	−8.25
13	99.51	90.21	91.88	−8.55
14	100	86.22	89.99	−8.1
15	99.77	90.83	92.23	−8.35

[a] Intraocular suitable particles: particles with sizes from 1 to12 μm.
[b] Vancomycin released from microparticles after 24 h of magnetic stirring in BSS.

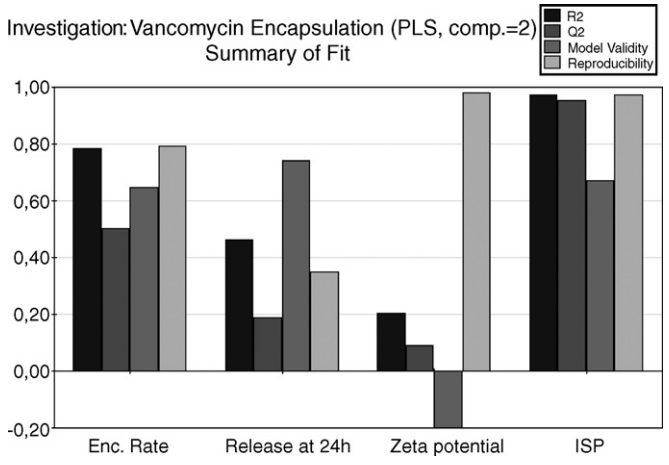

Fig. 1. Summary of fit showing the measure of fit (R^2), model predictability (Q^2), validity and reproducibility for each of the four studied responses: encapsulation rate, vancomycin released at 24 h, zeta potential and percentage of intraocular suitable particles (particles with sizes from 1 to 12 μm).

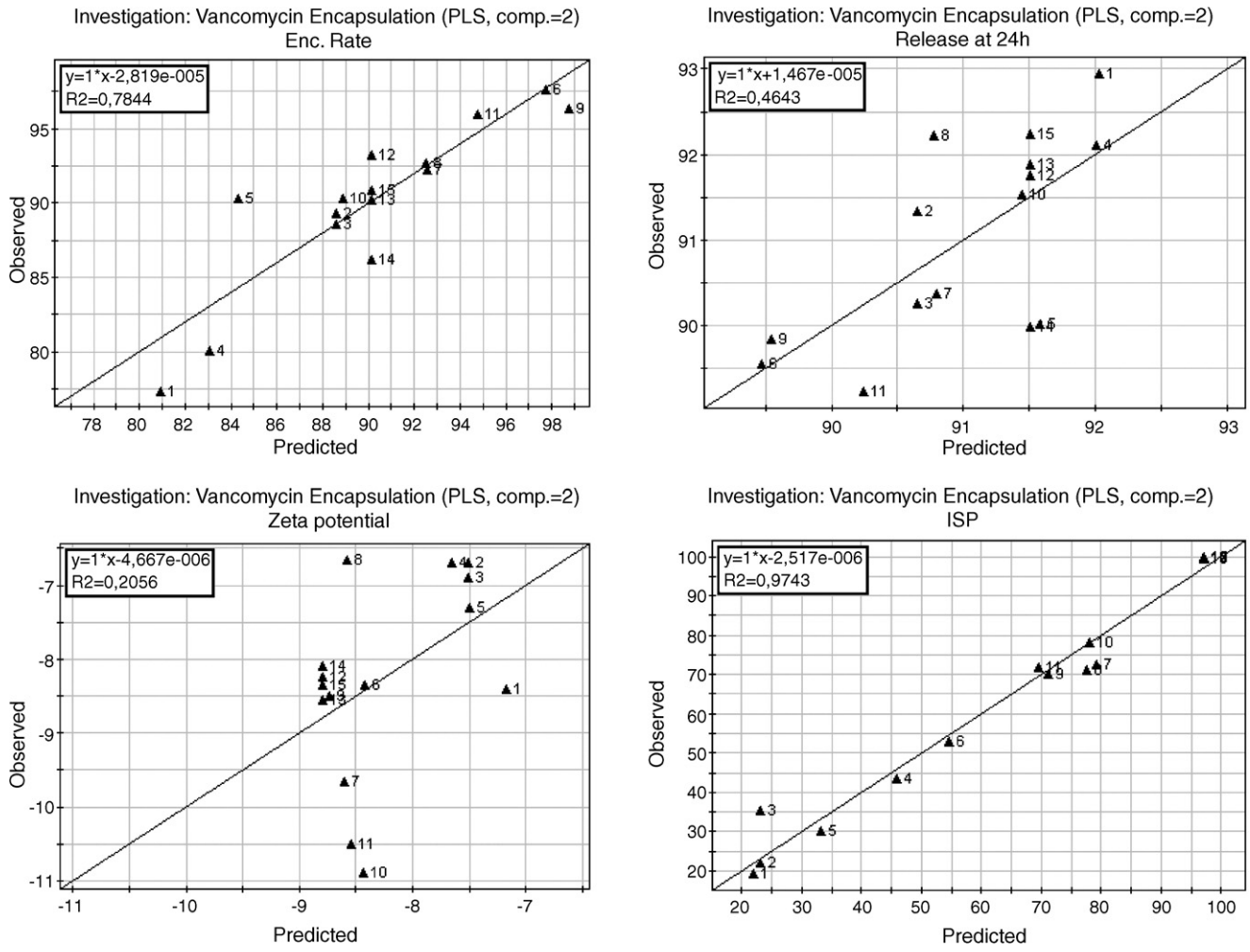

Fig. 2. Predicted versus experimental data for each of the four studied responses: encapsulation rate, vancomycin released at 24 h, zeta potential and percentage of intraocular suitable particles (particles with sizes from 1 to 12 μm).

correlations for these two responses were the following:

$$\text{ISP}\ (\%) = 91.23 - 4.19\text{Tm} + 19.36\text{Spd} - 14.83\text{Tm}^2 - 14.04\text{Spd}^2 - 1.69\text{Tm} \times \text{Spd}$$

$$\text{Encapsulation rate}\ (\%) = 5.53 + 2.08\text{Tm} + 2.32\text{Spd} + 2.65\text{Tm}^2 + 4.47\text{Spd}^2 + 2.12\text{Tm} \times \text{Spd}$$

where Tm was mixing time and Spd was mixing speed.

Normalized coefficient plot (Fig. 3) showed that mixing speed and time were important factors in determining particle sizes. These two factors were related to the second mixing process, which influenced droplet sizes in the final emulsion (double emulsion). The size of these droplets determined directly the final size of particles, since particles were immediately formed after solvent extraction and polymer solidification at the interface. Fig. 3 showed that increasing mixing speed and decreasing mixing time results in improving the fraction of particles having sizes between 1 and 12 μm (ISP). The response surface plot (Fig. 4) showed however an optimum mixing duration, around

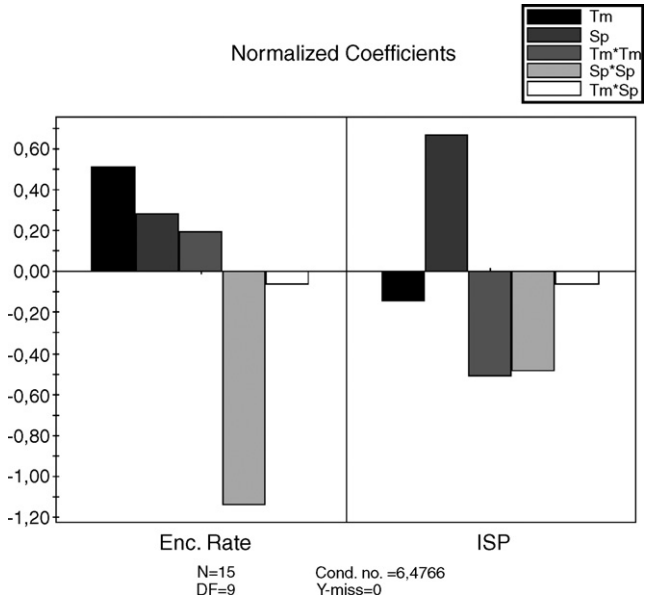

Fig. 3. Normalized coefficients plot for encapsulation rate and intraocular suitable particles proportion.

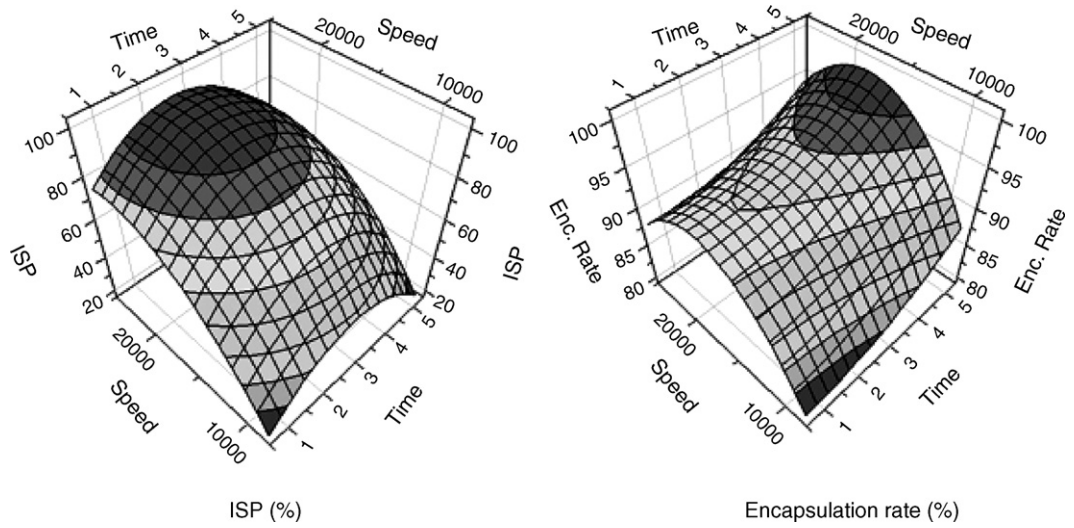

Fig. 4. Response surface plot for the proportion of intraocular suitable particles and encapsulation rate.

2.75 min. This phenomenon was simply due to the fact that the decrease in particle sizes, resulting from prolonging mixing duration, improved the ISP, by decreasing the population of the particles larger than 12 μm. Passing the optimum time, the loss in ISP was mainly due to the further decrease in size of the smallest particles, extending the population of particles below 1 μm.

Regarding encapsulation rate, the two studied factors had also a significant effect according to Fig. 3. The response surface plot (Fig. 4) showed this time an optimum mixing speed, around 18,000 rpm, over which increasing speed resulted in decreasing encapsulation rate. This is easily understandable given that high speed mixing could break the primary emulsion droplets and cause then a vancomycin transfer from internal to external aqueous phase, leading to a loss of vancomycin encapsulation.

As to the zeta potential, the larger its absolute value is, the more likely the suspension is to be stable, since the charged particles repel one another and thus overcome their natural tendency to aggregate. The zeta potential measurements showed negative charged particle surfaces, varying from −6.7 to −10.9 mV. All the obtained values were then acceptable and favoring a good stability. The zeta potential may then be safely discarded from the optimization step.

Regarding the release profile, the fraction of vancomycin released over 24 h varied from 89.22 to 92.95%. In endophthalmitis prophylaxis, we have to guarantee a minimum intraocular vancomycin charge over the first 24 h following cataract surgery. This meant that, assuming the release kinetics follow zero order, and for evident economic reasons, ideally all entrapped vancomycin has to be released over the first 24 h. This objective was nearly reached in all our experiments, and even if we were unable to find out a consistent model to optimize this kinetic, we believe all the obtained release profiles were economically acceptable.

3.2. Optimization

The aim of our work was of identifying the optimal process parameters in order to prepare microparticles suitable for intraocular application. Little data is available in literature about particle size constraints with respect to this route. Although a large range of particle sizes has been reported to be used in the intraocular route (Moritera et al., 1992; Wiechens et al., 1998; Yeh et al., 2001), we believe that particles must be larger than 1 μm and smaller than 12 μm. In fact, particles smaller than 1 μm will easily pass through the trabecular filter (Saraux, 1983), thus failing to stay long enough to deliver the drug into the anterior chamber of the eye. On the other hand, large particles might disturb the clarity of vision (Ackerman, 1962).

To take into account the interaction between factors and their complex influence on responses, a balanced protocol had to be fixed for optimum particle sizes and encapsulation rate.

Final process parameters were fixed using Modde sweet spot (Fig. 5). We targeted maximum ISP and encapsulation rate (giving more importance to sizes). Process parameters were as follows: Tm = 3.0167 min, Spd = 20500 rpm. Expected and

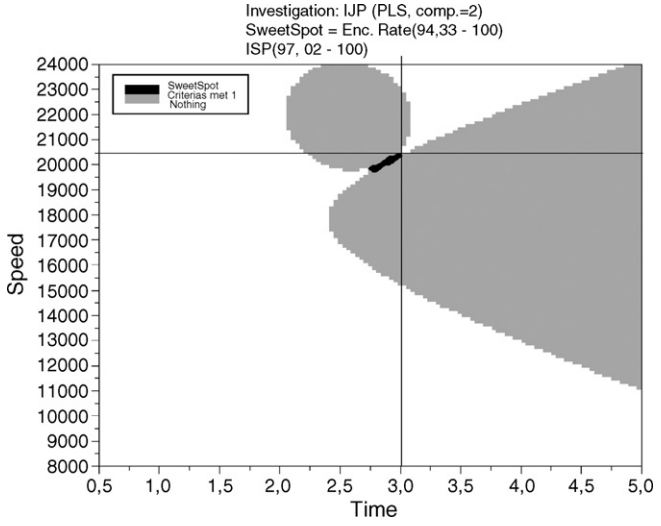

Fig. 5. Modde sweet spot. The sweet spot area corresponds to the combinations of speed and time leading to optimal responses.

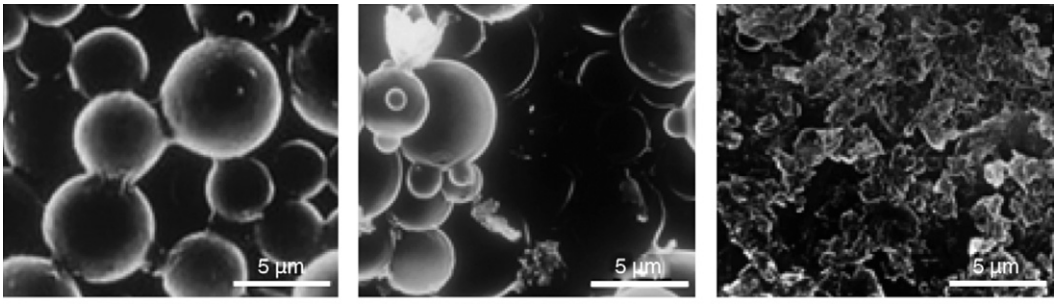

Fig. 6. SEM micrograph of optimized particles.

obtained responses were, respectively, 97.02 and 100% for ISP, 94.33 and 93.47 for encapsulation rate.

3.3. Final particles characterization

Fig. 6 shows the morphological features of PLGA drug loaded microparticles in SEM. The particles were perfectly spherical, with a smooth surface of polymer layers and no craters. The formation of some agglomerates is observed.

After incubation in the release medium for 2 weeks, only a small proportion of the particles had degraded, leading to multi-shaped polymer particles visible along with the drug loaded microparticles in the SEM photomicrograph. Release process was then not due to vancomycin liberation after polymer hydrolysis, since most of the antibacterial is released in the first 24 h, whereas particles needs much more time to degrade. After 8 weeks, nearly all the microparticles lost their form completely.

These observations confirm that large particles are likely to disturb patient vision for a long term, since full polymer hydrolysis needs weeks to occur.

The zeta potential measurements showed a negative surface charge. Zeta potential measured after particles fabrication was −7.6 mV. It remained negative and was −7.4 mV after 2 weeks.

Laser diffraction size measurements were carried out on samples of microparticles in suspension. Results in Fig. 7 showed that particle sizes vary between 1.6 and 11.8 µm.

Particles subjected to 2 weeks of mild mixing in BSS solution showed no significant increase in size distribution (sizes varied from 1.8 to 12 µm), that could result in a disturbance of vision. This stability in particle sizes may be partly explained by the negative zeta potential.

In vitro release profile of vancomycin from microparticles is shown in Fig. 8. Most of the vancomycin was released in the first 24 h (91.54%). Two steps were observed. A fast release step was observed during the first hour (release of 30.84% of encapsulated vancomycin). This phenomenon known as "burst effect" was caused by the immediately available drug fraction adsorbed to external particle surface and included in the matrix. Then a slower step occurred and lasted for 23 h (60.70% released). Extending the study for an additional 72 h showed that 3.7% of vancomycin was released over this period.

This profile showed that the fabricated particles are potentially usable in endophthalmitis prophylaxis at the end of cataract surgery. In fact, vancomycin release occurred to a large extent over the first 24 h. Particles are then likely to deliver sufficient antibacterial to maintain the needed concentration, which is a decisive improvement with respect to simple vancomycin solution (Gavini et al., 2004; Kodjikian et al., 2005). Indeed, it was demonstrated in a recent study that vancomycin used at 20 µg/mL along with irrigating solutions does not remain in the anterior chamber long enough to develop any bactericidal effect, due to its short intraocular half life (Kodjikian et al., 2005). Even if it initially reduces bacterial adhesion, it could result in a secondary increase of the adhesion of slime-producing bacteria.

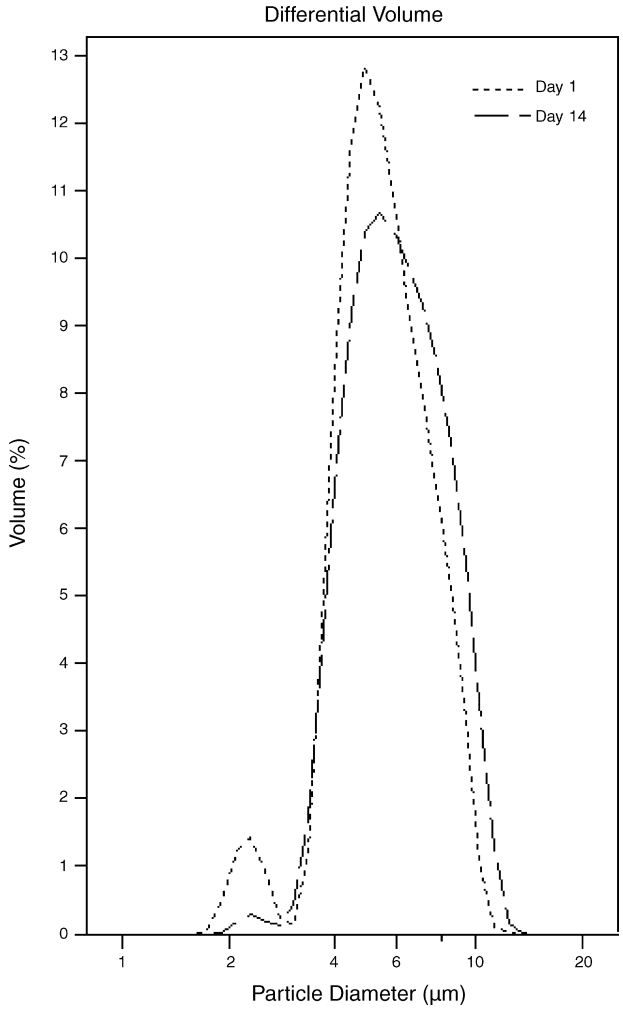

Fig. 7. Particle size distribution of optimized particles at days 1 and 14.

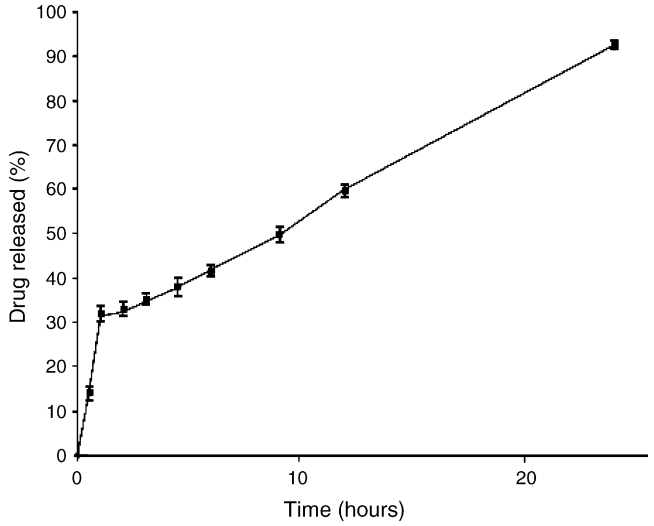

Fig. 8. Profile of vancomycin *in vitro* release from optimized microparticles in BSS solution and under mild magnetic stirring.

4. Conclusion

Vancomycin loaded microparticles are reported in literature for possible intrathecal and external ophthalmic (only at the ocular surface) use (Zimmer and Kreuter, 1995; Ozalp et al., 2001; Gavini et al., 2004), but never for intraocular application to our knowledge.

In the present study, we succeeded to prepare vancomycin loaded microparticles, with a satisfying encapsulation rate, and a suitable size, stability, and release profile for endophthalmitis prophylaxis after cataract surgery.

We were able to control particle sizes and encapsulation rate with both good model reproducibility and validity. We were not able to control zeta potential and vancomycin release profile, but in all our experiments, these two responses were good and suitable for our application. Experimental design is not common in the formulation of microparticles, however, our original approach showed that it can be an efficient method in controlling particle sizes and encapsulation rate, especially for the intraocular route, where size is important.

A convenient intraocular vancomycin concentration could be obtained *in vitro* by the present new microparticles, and thereby theoretically overcome the drawback of a short half-life within the anterior chamber. Anti-adhesion and bactericidal action of vancomycin microparticles remains of course to be evaluated in experimental and then clinical animal and human studies.

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