Factorial design optimization of tripalmitin based lipospheres for biopharmaceutical applications.

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Introduction

Every year severe and chronic diseases afflict millions of people and are becoming more prevalent as the population ages. In many case drug treatments for these diseases offer only limited therapeutic benefits and cause adverse side effects. For these reasons nowadays the worldwide market is interested to the development of advanced methodologies based on micro- or macroarrays and biosensors. This new technology has several advantages compared with conventional techniques; these include minimal reagent consumption, high sample throughput, ease of use, rapid chemical kinetics and high reproducibility, finding applications in several strategic areas such as predictive oncology, diagnostics in the biomedical field, and drug research (Bertucci et al., 2003; Borgatti et al., 2005; El-Ali et al., 2006). We are presently investigating the performances of a Lab-on-a-chip device, called DEParray™Chip (Manaresi et al. 2003), equipped with 102,400 arrayed electrodes. This Lab-on-a-chip is able to generate more than 10,000 indipendent dielectrophoretic (DEP) cages, where cells and microparticles can be immobilized. Inside the chip, the movement of the cages can promote the interaction between cells and microparticles, with successive separation. Among the different microparticulate system that can potentially fit to Lab-on-a-chip, we choose to study the applicability of lipid microspheres. Lipospheres (LS) consist of solid microparticles with a mean diameter usually comprised between 0.2 and 500 μm, constituted of a mixture of neutral lipids (generally triglycerides), where the bioactive compound(s) is dissolved or dispersed (Cortesi et al., 2005). LS present several advantages such as the high drug loading capacity, the good physical stability, the low cost of ingredients and the ease of preparation; so lipid microspheres combine the advantages of polymeric particles, fat emulsion and liposomes, avoiding some of their typical disadvantages such as cytotoxic effects after phagocytosis, toxic effects of organic residues after the production of polymers and lack of large industrial-scale production (Reithmeier et al., 2001).
In this work were described the optimization, by factorial design (Eriksson et al., 2000), of the preparation procedure of lipospheres and their application on a Lab-on-a-chip platform (DEParray™Chip).

Materials and methods

Glyceryl tripalmitate (GTP) and the dye Oil Red O were purchased by Fluka Chemical Co (Buchs, Swizerland), glyceryl monostearate (GMS) was a gift from Gattefossé (Saint-Priest Cedex, France) and polyvinyl alchol Celvol205® (PVA) was from Celanese chemicals Europe GmbH (Kronberg, Germany).
The preparation of cationic lipospheres was performed using the melt dispersion technique. A variable amount of a lipid mixture (GTP/GMS % 90/10 to 70/30) was melted at 70°C and then emulsified into 15 ml of an external aqueous phase containing 0.5-2% (w/v) of PVA as the dispersing agent. The emulsion was stirred using an IKA T25 Ultra-turrax (IKA Labortechnik, Germany) at 8000 rpm for 2 min. After stopping the agitation, the milky formulation was rapidly cooled to about 10°C by immersing the preparation in a cool water bath. After microparticles isolation by centrifugation (Sigma 2-16 centrifuge, Germany) the morphology and size distributions

of LS were evaluated by stereoscopic microscopy (Nikon SMZ 1500, Nikon Instrument S.p.a., Italia).
The experimental design and the evaluation of the experiments were performed by the PC software MODDE 8.0 (Umetrics AB, Sweden).
Manipulation of lipid microparticles was carried out in a DEParray™ Chip (Silicon Biosystems, Bologna, Italy).

Results and discussion

For studying the effect and the influence of different experimental parameters (i.e. the quantity of glyceryl monostearate respect to glyceryl tripalmitate, the PVA concentration and the oil to water ratio quantity) on the dimensional and morphological characteristics of lipospheres, a randomized central composite face-centered design (CCF) consisting of 17 runs was used. The parameters were varied as reported in the experimental matrix (Table 1).

Factors		
GMS quantity (% w/w)	PVA concentration (% w/v)	Oil to water ratio (%, w/w)
10	0.5	0.025
30	0.5	0.025
10	1.5	0.025
30	1.5	0.025
10	0.5	0.055
30	0.5	0.055
10	1.5	0.055
30	1.5	0.055
10	1	0.04
30	1	0.04
20	0.5	0.04
20	1.5	0.04
20	1	0.04
20	1	0.055
20	1	0.04
20	1	0.04
20	1	0.04

Table 1a. Experimental design factors matrix.

Responses	
Mean diameter (μm)	Standard deviation (μm)
9.8	6
9	5
13.4	6.8
11.2	5.6
13.6	9
10.4	5.6
15.8	9.2
11.3	6.4
13.5	7.4
10.7	5.3
13.8	6.7
10.6	6.2
12.6	6
12.8	6.7
8.5	5.8
9.8	6
10.9	6.3

Table 1b. Experimental design results matrix.

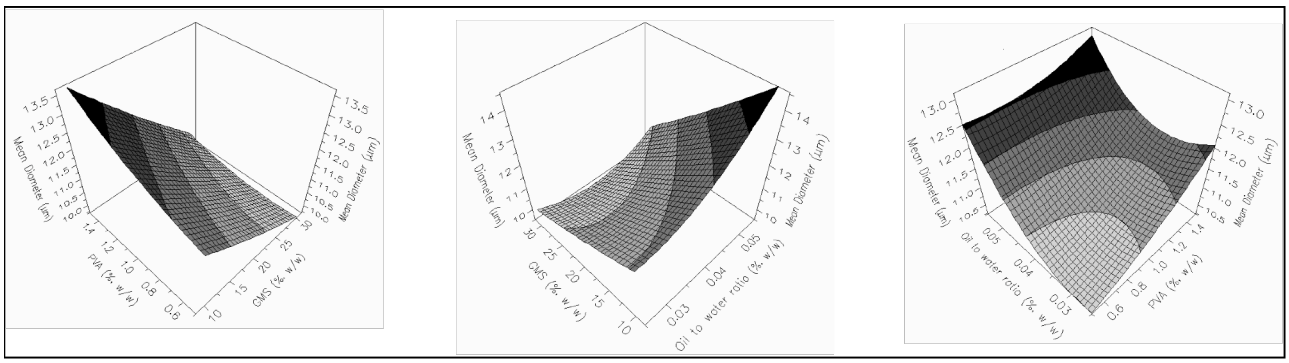

Fig. 1. Response surface plots from the factorial design showing the effect on lipospheres size (mean diameter) as a function of GMS quantity and PVA concentration (left), oil to water ratio and GMS quantity (middle) and PVA concentration and oil to water ratio (right).

Observing the results reported in Table 1 and the three-dimensional graphs illustrated in Fig. 1 it is evident that the parameter more influencing the mean particle size and the standard deviation is the oil to water ratio, while that one that had less importance is the concentration of the stabilizer (PVA).

For the determination of the performances (organization and separation) a DEParray™Chip consisting of 320x320 arrayed electrodes (each electrode measures 20x20 µm), generating more than 10,000 spherical DEP cages (Manaresi et al., 2003). Thanks to the small pieces of the electrodes single or small groups of microspheres (2-4 particles) can be trapped in separate cages and independently moved on the device (Fig. 2).

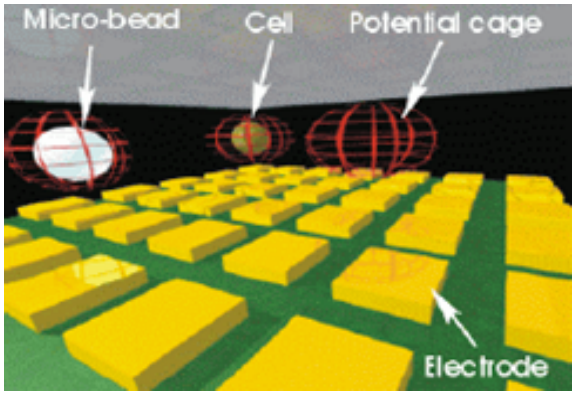

Fig. 2. Schematic representation of DEP cages in DEParray™ Chip.

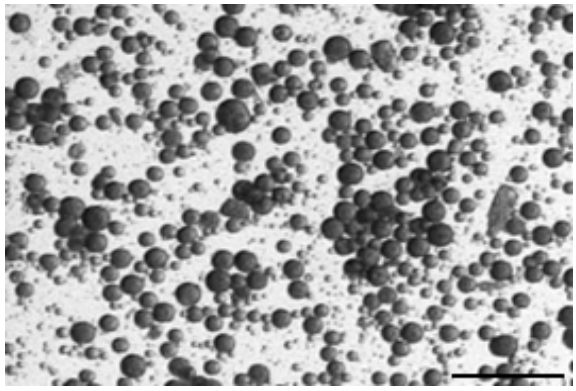

Fig. 3. Stereomicrophotograph of lipospheres prepared with GTP/GMS 80/20, 1% PVA and 0.04 of o/w ratio. Bar corresponds to 200 µm.

For the liposphere experiments were actuated two types of pattern, under software control: one with DEP-based cylinder cages separated by a gap of three row (pattern row1gap3) (Fig. 4-A) and one with DEP-based spherical cages separated one-by-one by three gaps array (pattern cage1gap3) (Fig. 4-B). In panel C and D of Fig. 4 were displayed the forced interaction (C) and subsequently the diagonal separation (D) between two lipid microparticles.

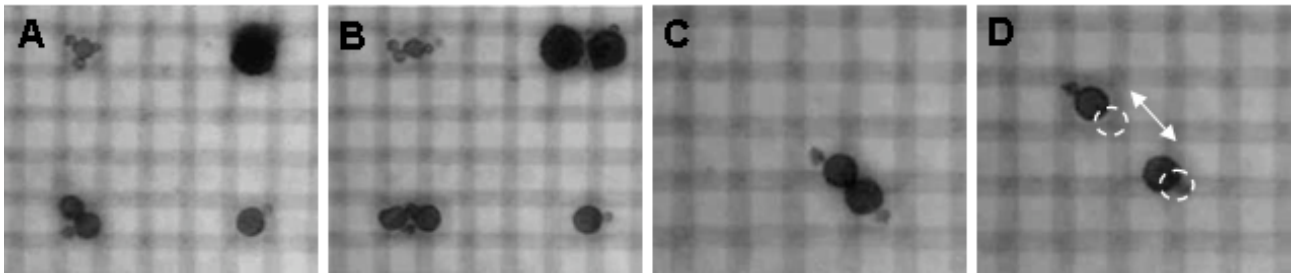

Fig. 4. Microphotographs showing the manipulation of lipospheres on DEParray™ chip. Organization in pattern1 cage3 (A) and pattern row1 cage3 (B); diagonal separations (C-D). The dashed circles represent the initial position of the lipospheres before the movement.

Conclusion

The results obtained by the factorial design approach, applied for the optimization and the screening of the experimental parameters, clearly shown that the parameter more influencing the mean particle size and the standard deviation is the oil to water ratio. It has also been observed that, as the dimension of microparticles intended for DEParray™Chip application should be between 1 and 20 µm (due to the dimensional constrains of the chip), all the lipospheres prepared could be suitable for this microsystem. Finally it has been demonstrated that our lipospheres can be efficiently loaded and manipulated on DEParry™Chip.

References

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