P-080 **Oral administration of insulin: Study of effect of different synthesis methods on size nanoparticles and their biological activity.**

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INTRODUCTION AND OBJECTIVES

Insulin, hypoglycaemic hormone, is administrated in diabetic patients by subcutaneously injection often painful and constraint for them. In the case of oral administration of insulin, a new complex pharmaceutical vector was developed. This complex vector is notably constituted by nanoparticles containing insulin confined into a vehicle (Frère Y. et al, CNRS Patent N° WO2004096172) and it's on theses nanoparticles that the study has focused.

Nanoparticles synthesis

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides, synthetic polymers... The selection of materials depend of many factors (Kreuter 1994) as size of nanoparticles desired; inherent properties of drug (solubility, stability...); surface characteristics (charge...); degree of biodegradability, biocompatibility and toxicity; drug release profile...

Nanoparticles can be prepared by several methods like dispersion of preformed polymers (Mohanraj 2006). In this case, nanoparticles are synthesized from biodegradable polymers like poly (D, L-lactide-co-glycolide) acid (PLGA) (Kompella 2001). This technique can be associated with solvent evaporation method where organic solvent (ethyl acetate) is used to dissolve polymers. The hydrophilic drug is emulsified into polymer to obtain a first emulsion (w/o), then this emulsion is added to an aqueous solution containing a surfactant or emulsifying agent to form double emulsion (w/o/w). After the formation of stable emulsion, organic solvent is evaporated. These emulsions can be obtained with different techniques as high-speed homogenization or ultrasonication.

The aim of this study is to compare size and biological activity of nanoparticles obtained by different synthesis methods. Three important parameters are studied: agitation technique, nature and concentration of surfactant.

MATERIALS AND METHODS

Nanoparticles containing insulin synthesis

Nanoparticles are synthesized by dissolving poly (D, L-lactide-co-glycolide) acid 50/50 from Boehringer Ingelheim in ethyl acetate and mixing it with Pluronic F68 (BASF) dissolved in same solvent. Then an aqueous solution of insulin (Umuline©, Eli Lilly) is dispersed into polymers solution by different techniques of homogenization (high speed homogenization or ultrasonication). The first emulsion obtained, a water/oil emulsion (w/o) is then dispersed in surfactant solution to obtain a second emulsion w/o/w by different techniques of homogenization. Then this multiple emulsion is added to a diluted solution of surfactant used in the second emulsion and solvent is evaporated by slow magnetic stirring (130rpm) overnight. The different modified parameters are:

- *Nature of surfactant agent for second emulsion and evaporation*: polyvinylic alcohol 18-88 (PVA) from Sigma-Aldrich or Pluronic F68.
- *Concentration of surfactant agent for second emulsion*: 2.5% or 3% of PVA 18-88.
- *Speed of agitation* in case of use of high speed homogenization (Ultra Turrax, UT, from IKA).
- *Agitation technique*: high speed homogenization for 60s at 6500rpm (first emulsion) and 24000rpm (second emulsion) or ultrasonication (US) for 15s at 33% (Bandelin) then 10s at 33% for first and second emulsion.

Characterisation of nanoparticles

Nanoparticles obtained were then diluted at 1/500 in ultra pure water in disposable cuvette placed in Zetasizer nano zs apparatus (Malvern Instrument) for size determination by dynamic light scattering; or diluted at 1/100 in 1mmol/L of NaCl in special cuvette for zeta potential measurement. Three lectures of each sample are done and each condition is done in triplicate.

RESULTS AND DISCUSSION

The reference technique for this study is high speed homogenization with a speed of 6500rpm for first emulsion and 24000rpm for the second emulsion (60s). For the evaporation, PVA 18-88 is used at 0.15%.

Influence of nature of surfactant

When PVA is replaced by Pluronic F68 for second emulsion and evaporation step, size particles is average of 2µm and zeta potential is strongly negative (table 1). Pluronic F68 doesn't provide small particles in this case.

Table 1: Size and zeta potential of nanoparticles synthetized with two different surfactants.

	Nature of surfactant	
Second emulsion Concentration	PVA 2.5%	Pluronic F68 2.5%
Evaporation Concentration	PVA 0.15%	Pluronic F68 0.15%
Size (nm)	384.3 ± 30.9	1985.2 ± 2496.4
Zeta potential (mV)	-1.9 ± 0.1	-53.6 ± 1.3

Influence of concentration of surfactant

For the second emulsion, concentration in surfactant PVA 18-88 seems to reduce size nanoparticles from ~384 to ~300nm without modifying zeta potential (table 2). More PVA is concentrated, more viscous is the solution, this could explain results obtained.

Table 2: Size and zeta potential of nanoparticles synthetized with different concentrations of PVA.

	Concentration of PVA	
Second emulsion	2.5%	3%
Size (nm)	384.3 ± 30.9	300.9 ± 31.4
Zeta potential (mV)	-1.9 ± 0.1	-2.0 ± 0.1

Influence of speed agitation with UT

Results show that agitation speed play an important role in size determination of nanoparticles but not in zeta potential (table 3). For nanoparticles synthesized with a slower speed for first emulsion, bigger size is obtained (~384nm) contrary to other samples where first emulsion speed is at 24000rpm and size nanoparticles near 245nm. However, agitation speed in second emulsion doesn't seem to modify size. Speed of first emulsion is critic to obtain small nanoparticles.

Table 3: Size and zeta potential of nanoparticles synthetized with UT at different speeds.

	Agitation speed for 60s (rpm)		
First emulsion	6500	24000	24000
Second emulsion	24000	24000	6500
Size (nm)	384.3 ± 30.9	248.3 ± 9.6	242.5 ± 13.4
Zeta potential (mV)	-1.9 ± 0.1	-2.7 ± 0.3	-2.3 ± 0.4

Influence of agitation technique

Results presented above in table 4 show that only ultrasonication, in both emulsions, permit to obtain smaller nanoparticles. When US is replaced by UT in second emulsion, bigger size is obtained (~265nm) but smaller than use of UT in both emulsion (~384nm).

Table 4: Results of size and zeta potential of nanoparticles synthetized by different techniques of agitation.

	Agitation techniques		
First emulsion	UT 60s 6500rpm	US 15s 33%	US 15s 33%
Second emulsion	UT 60s 24000rpm	UT 60s 24000rpm	US 10s 33%
Size (nm)	384.3 ± 30.9	265.3 ± 10.1	168.1 ± 6.1
Zeta potential (mV)	-1.9 ± 0.1	-2.7 ± 0.5	-5.5 ± 2.2

Effect of size nanoparticles on biological activity

First results obtained with nanoparticles (NP) containing Umuline in diabetic rats show that insulin encapsulated in NP is significantly more active than Umuline alone at the same concentration of insulin (figure 1): normoglycemia is reached faster (9h).

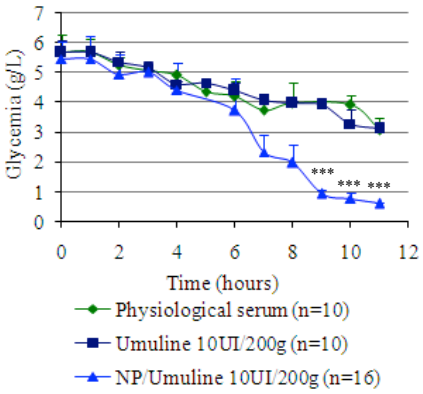

Figure 1: Effect of nanoparticles/Umuline synthesized with US and administrated by intra-duodenal injection on glycemia of diabetic rats.

CONCLUSIONS

This study shows that only US give very small size of nanoparticles. In the case of UT, a big agitation speed for first emulsion and the increase of concentration of PVA in second emulsion permit to obtain smaller size nanoparticles. This study has to continue by varying agitation conditions and surfactant to obtain size nanoparticles as small as these obtained with US. Finally, biofunctionality of nanoparticles obtained by UT will be verify in *in vivo* on diabetic rats.

REFERENCES

- Frère Y., Danicher L., Belcourt A. (2004) *Vecteur pour administration par voie orale.* CNRS Patent N° WO2004096172.
- Kreuter J. (1994) *Nanoparticles* in *Colloidal drug delivery systems*, Editor Marcel Dekker (New York, US) 219-342.
- Mohanraj VJ. Et al. (2006) *Nanoparticles – A review.* Trop J Pharm Res, 5 (1) 561-573.
- Kompella UB. et al. (2001) *Poly (lactic acid) nanoparticles for sustained release of budesonide.* Drug Deliv. Technol. 1: 1-7.