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Production of lipophilic nanogels by spontaneous emulsification

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SD and BM have contributed equally

Abstract

Nanosized gel particles, so-called nanogels, have attracted substantial interest in different application fields, thanks to their controllable and three-dimensional physical structure, good mechanical properties and potential biocompatibility. Literature reports many technologies for their preparation and design, however a recurrent limitation remains in their broad size distributions as well as in the poor size control. Therefore, the monodisperse and size-controlled nanogels preparation by simple process –like emulsification– is a real challenge still in abeyance to date. In this study we propose an original low energy emulsification approach for the production of monodisperse nanogels, for which the size can be finely controlled in the range 30 to 200 nm. The principle lies in the fabrication of a direct nano-emulsion containing both oil (medium chain triglycerides) and a bi-functional acrylate monomer. The nanogels are thus formed *in situ* upon UV irradiation of the droplet suspension. Advantage of such modification of the oil nano-carriers are the potential modulation of the release of encapsulated drugs, as a function of the density and/or properties of the polymer chain network entrapped in the oil nano-droplets. This hypothesis was confirmed using a model of hydrophobic drug – ketoprofen– entrapped into the nanogels particles, along with the study of the release profile, carried out in function of the nature of the monomers, density of polymer chains, and different formulation parameters.

1. Introduction

Nanogels can be defined as a three-dimensional cross-linked polymer network dispersed as nanoparticle and swollen by a liquid phase. Nanogels have received considerable attention in many aspects due to their potential applications such as sensing (Peng et al., 2010), diagnostics (Oishi et al., 2007, Hasegawa et al., 2005), bioengineering (Hayashi et al., 2009) and drug delivery (Qiao et al., 2011). In contrast with conventional polymer nanoparticles which exhibit a packed matrix structure, nanogels exhibit a much higher loading capability, and can be considered as hybrid cargo between emulsions, capsules, and polymer nanoparticles. Molecules of interest can be trapped inside the gel swelling phase and, according to the properties of the polymer network their delivery can be tuned and finely controlled. Nanogels have been formulated by various strategies based on the gelation of polymers already solubilized in the dispersed phase, by either covalent or non-covalent cross-linking. The first approach covalent cross-linking results in the gelation of nano or macro-emulsion (McAllister et al., 2002), fabrication of polymerized block copolymer micelles (Chacko et al., 2012) or nano-template method in which the particle size is controlled by well-defined templates. (Sasaki and Akiyoshi, 2010, Yan et al., 2017). The polymer chemistry was carried out through different ways, such as cross-linking on disulfide groups (Ryu et al., 2010b) and amide cross-linking (Zhuang et al., 2012) similarly to those existing in biological environment. On the other hand, nanogels were also fabricated through polymer cross-linking by non-covalent interactions, such as hydrophobic interactions, hydrogen bonding, van der Waals interactions. (Raemdonck et al., 2009, Gruber et al., 2018, García and Cuggino, 2018). Importantly, most of the methods reported in literature deal with the fabrication of hydrogels or nano-hydrogels thus using water-soluble polymers (Ryu et al., 2010a, Torchilin, 2005). However, the encapsulation of hydrophobic and / or lipophilic molecules is only described in a few reports, in the case of micelle cross-linking (O'Reilly et al., 2006) or through self-assembly of amphiphilic polymers (Ryu et al., 2010b).

In this study, we developed a novel approach to formulate and synthesize lipophilic nanogels based on spontaneous emulsification of an {oil + monomer} phase, followed by in situ photopolymerization of the nano-droplets. The oil phase is composed of a mixture of a monofunctional monomers (ethyl acrylate, butyl acrylate), difunctional monomer (tri(propylene glycol) diacrylate, TPGDA), vegetable oil (medium chain triglycerides), and photoinitiators (detailed in the experimental section). The process was based on the fact that the

monomeric phase behaves very similarly as vegetable oils, as regards the spontaneous emulsification process (Anton and Vandamme, 2011, Wang et al., 2019). The spontaneous process is indeed related to the respective affinities between the oil phase, the nonionic surfactant, and the aqueous phase; in this study, TPGDA shows a behavior close to the one of oils, that allows to obtain nanogels with a controllable size and homogeneous size distribution. Herein we investigated the nanogels formation, the impact of the chemical and formulation parameters on suspension properties, and, on the second hand, the release profiles of model drug encapsulated. Ketoprofen as model of hydrophobic drug nonsteroidal anti-inflammatory having analgesic and antipyretic properties used in the treatment of rheumatic and non-rheumatic inflammatory disorders (Molina-García et al., 2013, Khan et al., 2013) was encapsulated and its controlled release investigated.

2. Experimental section

2.1. Materials

Medium chain triglycerides (MCT, Labrafac[®] WL 1349) was purchased to Gattefossé S.A., Saint-Priest, France. Tri(propylene glycol) diacrylate (TPGDA), ethyl acrylate (EA), *n*-butyl acrylate (nBA), 1-hydroxycyclohexyl phenyl ketone (HCPK, as photoinitiator, 99% purity) were obtained from Sigma-Aldrich. Water used was milliQ water, and a nonionic hydrophilic surfactant (Kolliphor ELP[®]) was purchased from BASF, Ludwigshafen, Germany. It is a polyethoxylated-35 castor oil with a Hydrophilic-Lipophilic Balance (HLB) of 12 ~ 14. Ketoprofen was obtained from S.I.M.S (Florence, Italy).

2.2. Preparation of nanogels

2.2.1. Nano-emulsification

The general principle of spontaneous nano-emulsification is the sudden mixing of an oil phase homogenized with hydrophilic surfactants, at elevated temperature with the aqueous phase at room temperature. As a result of the rapid change in the surfactant solubility between oil and water, the oil phase is broken-up and generate nano-emulsion. (Rehman et al., 2019) The water phase brutally integrates the oily one, fast enough to make the oil molecules to undergo a spinodal-like decomposition generating the oil droplets. (Miller, 1988) In this study, the oil phase was a mixture of MCT and TPGDA two components extremely miscible each other at different weight contents. The concentration of TPGDA in the oil phase was varied from

10 wt.% to 50 wt.%. Then, the formulation parameter relating the mixing proportions with the nonionic surfactant was noted surfactant-to-oil weight ratio, defined as $SOR = 100 \times w_{\text{surfactant}} / (w_{\text{surfactant}} + w_{\text{oil}})$, where w indicates the weight of the different compounds, was varied from 20% to 70%. Finally, the water content in all the formulations was kept constant at 60 wt.% of the final suspension. Once the {oil + surfactant} phase was mixed, homogenized (vortex), and set at 75°C, the aqueous phase at room temperature was suddenly added in it and the whole mixture was again homogenized (vortex) for one minute, up to the nano-emulsions was completely generated and homogeneous. Variations of the monomer composition were also studied, substituting a part of TPGDA for either *n*-butyl acrylate or ethyl acrylate (details below).

2.2.2. Polymerization in nano-droplets

As illustrated in Figure 1 (a) stable nano-emulsions were generated by a simple phase mixing (see sub-section above) and vortex phase homogenization. The freshly prepared nano-emulsions were polymerized by UV irradiation in a tube-in-tube device (Figure 1 (b)). The nano-emulsions were charged in a 10 mL plastic syringe (HSW, Norm-Ject, France), placed in the syringe pump (KDS LEGATO 200, KdScientific) holder, delivering the nano-emulsion through a 30 cm long polytetrafluoroethylene (PTFE) tubing (1.68 mm ID x 3.2 mm OD) at a constant flowrate of 63 μ L/min. The PTFE tubing was inserted inside a 4 cm ID stainless steel tube equipped at both ends with a T-junction (Swagelok) in which were set the two light guides of an UV source (Lightningcure LC8, Hamamatsu) operating at $\lambda = 365$ (nm) wavelength corresponding to the maximum of absorbance of the photoinitiator (HCPK) added into the nano-emulsion's monomer phase (3.5 wt.% of monomer phase). According to the size of tubing and flow rate, the residence time of the nano-emulsions under the UV irradiation was about 11 min. The final resulting polymerized nano-emulsion, i.e. nanogel was collected as homogeneous and clear sample, visually similar to common nano-emulsions

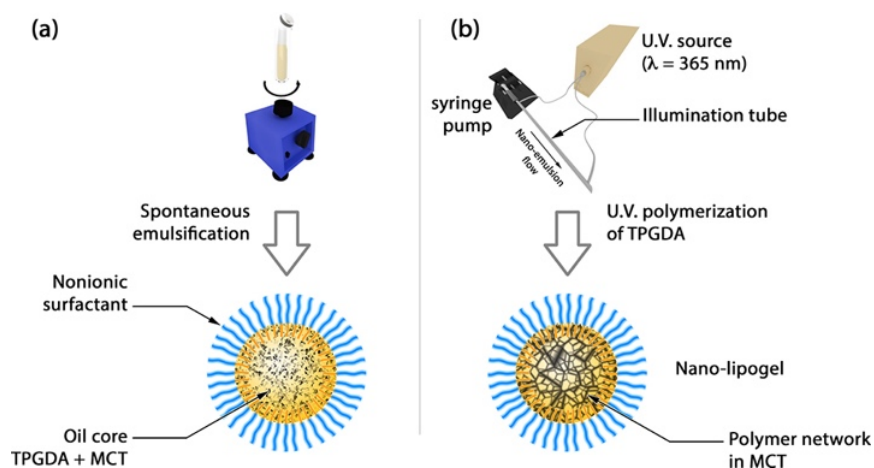


Figure 1: Schematic drawings of (a) the low-energy nano-emulsification of non-polymerized nano-droplets suspension, and (b) continuous-flow U.V. polymerization setup producing nanogels.

2.2.3. Preparation of ketoprofen-loaded nanogels

Ketoprofen-loaded nanogels containing a combination of difunctional and monofunctional monomers (TPGDA, EA and BA) were prepared by mixing the drug into the MCT (Labrafac[®] WL) at 0.5wt.%. While the impact of monomer concentration in oil monomer to oil weight ratio was varied and studied regarding the study of the formulation, in this stage of ketoprofen encapsulation and release, this ratio was chosen to be 50%, and SOR at 40%. The monomer phase was composed of pure TPGDA (50%) or a mixture of TPGDA (7.5 wt%) with either EA or BA (42.5 wt.%).

2.2.4. Dynamic light scattering

Hydrodynamic diameter, the size distribution and polydispersity indexes (PDI) of the nanogels suspensions were assessed by dynamic light scattering (DLS) using a Malvern Nano ZS instrument (Malvern, Orsay, France). The helium-neon laser (4 mW) was operated at 633 nm, the scatter angle was fixed at 173° and the sample temperature was maintained at 25°C. The PDI of the particle size distribution is a measure of the broadness of the size distribution and it is commonly admitted that PDI values below 0.2 corresponds to monomodal distributions. DLS measurements were performed in triplicates after dilution (at 1/50) in ultrapure water.

2.2.5. UV spectrophotometry for drug release measurement

Ketoprofen release studies were carried out with a dialysis tubing (regenerated cellulose, MWCO 12-14000 Daltons, Medicell International Ltd, London, U.K.). Two grams of freshly

prepared nanogel emulsions were charged into the dialysis tubing which was then immersed into 500 mL of Phosphate Buffer Solution USP (6.8pH) gently agitated by magnetic agitation at 250 rpm and (37 ± 1) °C. Samples of dialysate (2mL) were collected after predetermined time in such a way that this amount of dialysate was replaced by same amount of medium pre-warmed at 37°C to insure sink conditions. The concentration of ketoprofen in the dialysate was determined with UV spectrophotometer (Cary 4000-HP, Agilent technologies) operating at 260 nm, using a calibration curve beforehand established (with linear extrapolation fitting with: $Abs = C \times 0.0673 + 0.0011$, with Abs is the light absorbance and C the ketoprofen concentration in ug/ml, $R^2 = 0.999$, data not shown). Experiments were performed in triplicate.

It is important to note here that the drug release through a dialysis bag, in some conditions, can be shifted from the actual drug release from the drug carrier. As reported in a recent study (Yu et al., 2019) the difference between the actual drug release from the nanocarrier and the one in the release medium outside of the dialysis bag can be impacted by both the cut-off and the nature of the membrane. However, in the present study, the experimental conditions used, 12kDa and RC membrane, fit with the ones for which the drug release outside of the dialysis bag are almost superimposed with the actual drug release from its carrier.

3. Results and discussions

3.1. Impact of the formulation parameters on the nanogel size

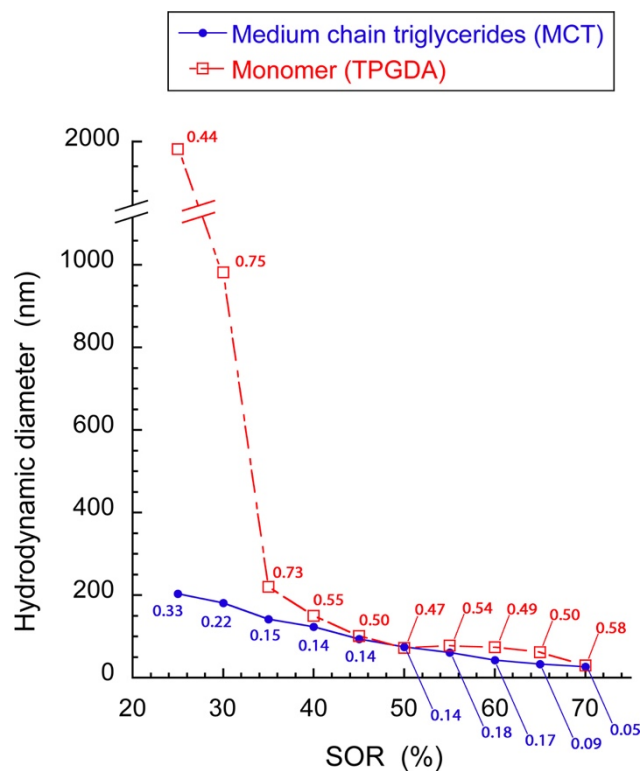


Figure 2: Impact of the surfactant amount (surfactant-to-oil weight ratio, SOR) on the size distribution of the nano-emulsions generated by spontaneous emulsification. Oil phase is either pure MCT (filled circles, Labrafac[®] WL), or pure monomer, TPGDA (open squares). The figures present mean hydrodynamic diameter and PDI (indicated as insets for each point) plotted against the surfactant amount (presented with the SOR parameter) (n = 3).

Low-energy nano-emulsification methods have been described through several methods like spontaneous emulsification or phase inversion temperature method which was recently shown to be based on similar mechanisms (Anton and Vandamme, 2009). In general, the formulation of oil-in-water emulsions is performed by suddenly mixing the oily phase containing a high-HLB non-ionic surfactant fully miscible in certain conditions of temperatures with the aqueous phase. The process is driven, after the phase homogenization, by the sudden change of the surfactant solubility. Since these PEGylated surfactants were first mixed in the oily phase, when these are homogenized with the aqueous one (for which solubility is better) (Anton and Vandamme, 2011), the water spontaneously penetrated and broke up the {oil + surfactant} phase, turning the oil phase into nano-droplets dispersed in the aqueous phase according to a spinodal-like decomposition (phenomenon we described in previous reports (Anton and Vandamme, 2009). Spontaneous emulsification takes benefit of the intrinsic physicochemical properties of the surfactants, co-surfactants and excipients in the formulation, to create the

nano-dispersion of the oily phase. As a result, the lipophilic compounds were homogeneously dispersed in the droplets, assuming that all the nano-droplets have the similar composition of MCT, monomer, co-monomer, initiator, etc. The polymerization step logically might give a population of similar droplets.

The first sets of results concerned spontaneous emulsification stage, and more particularly the outstanding emulsifying properties that can be obtained with the TPGDA monomer without oil or in combination with MCT, since these two components are extremely miscible. In the case of MCT / surfactant, as illustrated in Fig. 2 (filled circles), increasing the surfactant concentration classically resulted in gradually decreasing the droplet size, along with a rather good monodispersity *i.e.* PDI close to 0.15. The global sizes exhibited a monotonous downturn from 200 nm for SOR around 25 %, up to hydrodynamic diameters below 50 nm for SOR higher than 60 %. On the other hand, when all the MCT phase was substituted for the lipophilic monomer TPGDA (Fig. 2, open squares), the trend appeared similar for $SOR \geq 40\%$, with a size around 200 nm for the SOR value around 35 % and decreasing below 100 or 50 nm for $SOR > 50\%$. However, the dispersity appeared much higher with PDI higher than 0.4, indicating that the process worked in dispersing the monomer phase, but was definitely less efficiency than with vegetable oils and created disparate size distributions. The nano-emulsions obtained with pure monomers (Fig. 2, open squares), present indeed a poor monodispersity, denoting a significant behavior as compared to pure MCT. Considering the nano-emulsification of a mixture of MCT and TPGDA as oil phase in different weight ratios for different fixed SOR (from 20 to 60 %), the oil-to-monomer weight ratio impacted the size and PDI (Fig. 3). Regarding the effect of SOR on the droplet size and PDI, the trends were close and showed a decrease in size linked to the surfactant concentration. More interesting, the increase in TPGDA concentration appeared highly beneficial for the process, impacting the droplet size, but mostly improving the monodispersity of the suspension decreasing the PDI value below 0.09-0.08 in some cases as opposed to what was observed with TPGDA alone.

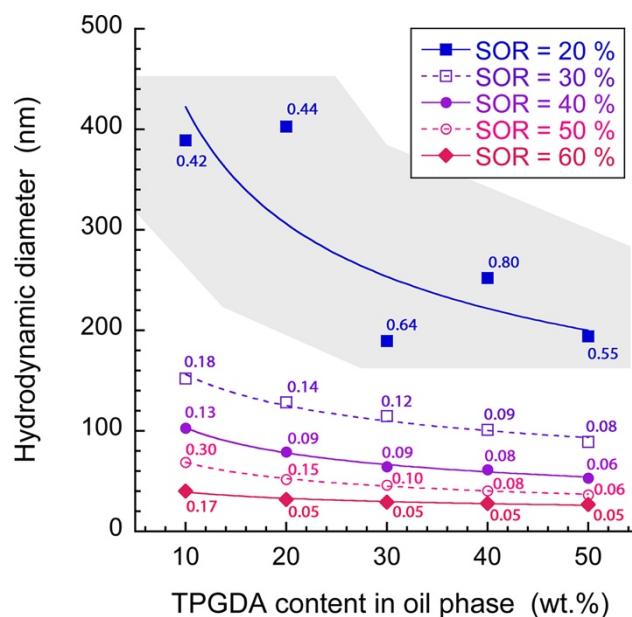


Figure 3: Nanogels formulated with the mixture of MCT and TPGDA. Hydrodynamic diameter and PDI are plotted against the TPGDA weight content, fit curves are power law, displayed on the figure to guide the eye ($n = 3$). The corresponding PDI values are indicated in the figure, and the gray light region shows the formulations (SOR = 20%) with high PDI values, indicating polydisperse samples, *i.e.* not fitting with the criteria of satisfactory formulations.

Mixtures of MCT and TPGDA allowed the production of smaller and more monodisperse droplets compared to pure MCT or pure TPGDA. For a SOR of 30%, the size decreased from 152 nm (PDI = 0.18) down to 100 nm (PDI = 0.08) for TPGDA content in MCT of 10 to 50 wt.%, respectively. In comparison, for the same SOR (30 %), the formulation with pure MCT gave 181 nm (PDI = 0.22), and even, with pure TPGDA the process did not work properly with size around 1 μm and PDI > 0.7 (*i.e.* DLS measurements does not have meaning). As regards the SOR = 60 %, the size remained constant over the change of composition of the oil phase below 50 nm, reaching around 35 nm for TPGDA content at 50 wt.% while it was about 40 nm and 75 nm when using pure MCT and pure TPGDA, respectively. These results emphasized that the mixture of MCT and monomer TPGDA improve the emulsification process as compared with formulations with individual pure compounds. Besides the current study, these results could find some interest in the optimization of the spontaneous nano-emulsification process. It is also important to note that at a too low surfactant concentration (SOR = 20%, light gray region in the figure), the spontaneous emulsification process is not working successfully, giving rise to highly polydisperse formulations, whatever the oil phase

composition. This shows that the most important factor impacting on the nano-emulsification process, in that case, is not the oil phase composition, but rather the surfactant concentration.

3.2. Drug-loaded nanogels

Such nanogels have a panel of potential applications in drug encapsulation and their release can be controlled by the gel structure and density. The tunability, encapsulation of model compounds, and monitoring of its release was investigated as a function of the gel characteristics. The nanogel consist of a cross-linked network of (poly(TPGDA), poly(BA) or poly(EA)) polymer chains swollen by MCT form bi-continuous structures able to modulate the release of compounds solubilized in the oil core of the lipid nano-droplet. As a proof of concept, nanogels have been designed to encapsulate a hydrophobic drug (ketoprofen, KP) by admixing the oil phase with the drug prior to spontaneous emulsification. By varying the weight content of the cross-linker (TPGDA) and type of the monofunctional monomer (EA or BA), one expects to modulate the drug release profile by changing either the density of the cross-linked polymer network (variation of the amount of cross-linker and polymerization time), or the interaction between the drug and monofunctional monomer. The drug release profile was determined by following the concentration of the ketoprofen in the dialysate by UV spectrophotometry. Ketoprofen exhibits an absorbance peak at 260 nm. However, it turned out that the monomers selected (TPGDA, EA and BA) also absorbed at that wavelength which may affect the determination of the drug release profile if unreacted monomers were present.

Therefore, a series of experiments without ketoprofen were conducted to establish for each monomer used its absorbance value at 240 nm with time following the same procedure described for the drug release. Formulation were composed of pure MCT containing different monomers (tri(propylene glycol) diacrylate or ethyl acrylate or butyl acrylate) along with hydrophilic nonionic surfactant Kolliphor ELP[®]. The monomer was used either alone or in combination with other monomers having different ratios in oil. The results depicted in Fig. 4 showed the KP release profiles according to the composition and the presence or absence of polymers, including experiments with monomer before polymerization compared to polymer nanogel after polymerization.

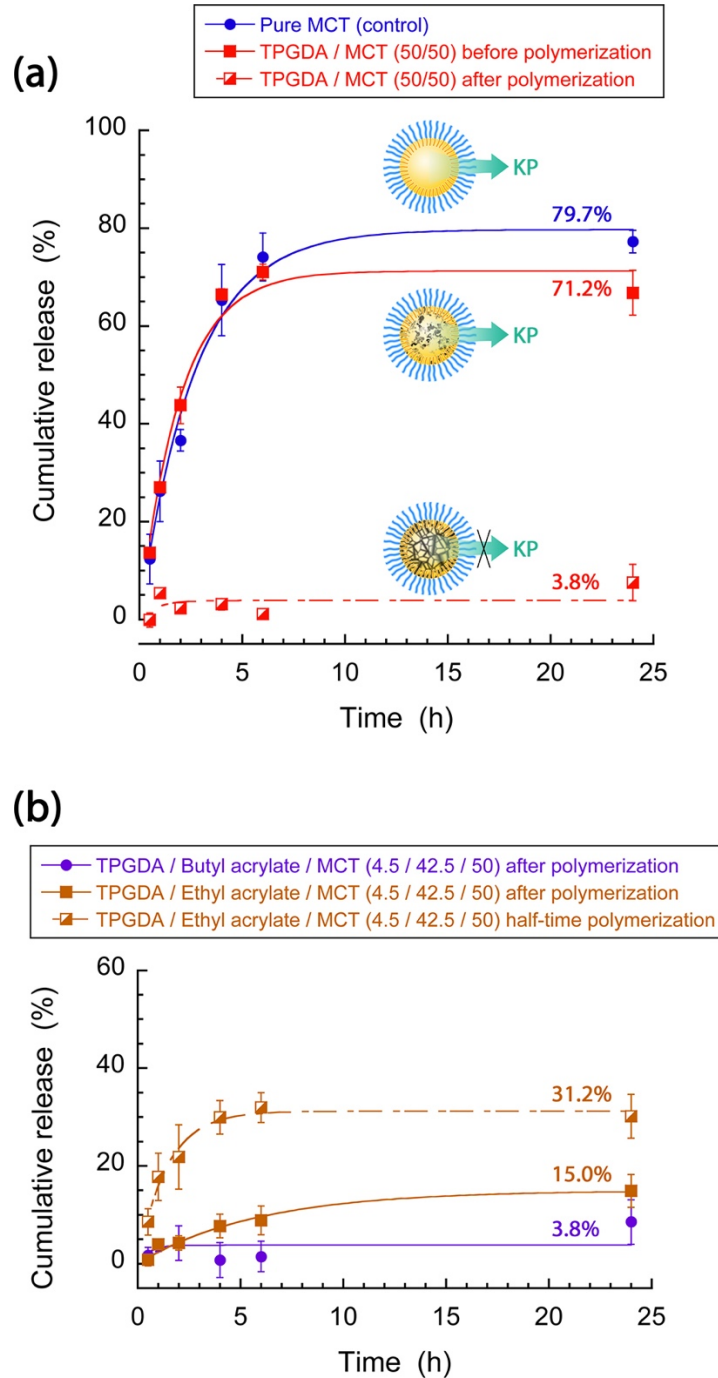


Figure 4: Release profiles in PBS of KP encapsulated in oil reservoir of nano-emulsions or nanogels, for different composition and experimental conditions (n = 3).

In order to ensure KP solubilization in the release medium USP phosphate buffer solution (pH = 6.8) was chosen. In Fig 4 (a), KP was encapsulated in nano-emulsion made with pure MCT, and a formulation composed of 50% of MCT and 50% of monomer, before and after polymerization. These three nano-suspensions were comparable in size and polydispersity, being 81 nm (PDI = 0.17), 89 nm (PDI = 0.19) and 99 nm (PDI = 0.20), respectively.

Regarding the release profiles, the curves exhibited, as expected, an exponential growth, showing a burst release and stabilizing as a plateau. The pure MCT and monomer samples actually showed a similar behavior with a significant release, giving rise to a plateau around 79% and 71%, respectively. The fact that cumulative release did not reach 100% implied that a part of the drug was still encapsulated in the oil core, likely a residual solubilization of the drug, which was shared between oil and water. On the other hand, the same sample after the creation of lipogel after polymerization of TPGDA totally inhibited the drug leakage making the cumulative release around 4% after 24h. This is a strong evidence of the advantages brought out by the lipogel in the formulation: the polymer network indeed retained the encapsulating molecules, as expected. Interestingly, owing to the fact that monomeric residues, in theory, can interfere with the absorbance of KP, the close location of the curves (Fig. 4 (a)) of the control in pure MCT and the mixture TPGDA/MCT (50/50) before polymerization shows that their impact on the methodology is negligible, likely explained by their poor solubility in the bulk aqueous phase.

In Fig. 4 (b), investigations were extended, replacing the major part of TPGDA by *n*-butyl acrylate or ethyl acrylate (in order to open the formulations to future applications to functionalize the polymer), providing, after polymerization, droplet size around 69 nm (PDI = 0.13) and 85 nm (PDI = 0.09), for ethyl acrylate and *n*-butyl acrylate, respectively. KP release results were comparable, for both samples after polymerization, lipogels formed in the nano-droplets induced an important drug retention –*i.e.* releases around 15% and 4% after 24h, respectively comparable to the one shown in Fig. 4 (a) with pure TPGDA. A last control was KP release when the polymerization was incomplete *i.e.* after only half of the time necessary for the full monomer conversion (*i.e.* 5 min). The results indicated an increase of the KP leakage, reaching around 30% after 24h, confirming the hypothesis and previous results.

4. Conclusion

The general objective of this study was to create a lipogel inside the droplet core in order to decrease or inhibit the premature release of encapsulated materials. To this end, a new and facile route to synthesize lipophilic nanogels swelled with MCT was proposed. The first step was the spontaneous emulsification of a difunctional monomer (TPGDA) / MCT (Labrafac[®]WL) oil phase, to form nano-emulsions. Then, the second step was the photopolymerization of the nano-droplets to obtain a colloidal nanogels dispersion. Results showed that this method can be used to produce monodisperse nanogels at low surfactant amount (*i.e.* low SOR), with a size easily

tunable from *ca.* 200 nm to *ca.* 30 nm according to the formulation parameters. The main advantage of nano-emulsions is their high loading capacity and we have shown in the last part of the study, that the nanogels inhibited the premature leakage of a model drug encapsulated (ketoprofen), from *ca.* 80% to 4% release after 24h, in absence or presence of the lipogel, respectively.

5. Acknowledgments

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6. References

- Anton, N., Vandamme, T.F., 2011. Nano-emulsions and Micro-emulsions: Clarifications of the Critical Differences. *Pharmaceutical Research* 28, 978–985.
<https://doi.org/10.1007/s11095-010-0309-1>
- Anton, N., Vandamme, T.F., 2009. The universality of low-energy nano-emulsification. *International Journal of Pharmaceutics* 377, 142–147.
<https://doi.org/10.1016/j.ijpharm.2009.05.014>
- Chacko, R.T., Ventura, J., Zhuang, J., Thayumanavan, S., 2012. Polymer nanogels: A versatile nanoscopic drug delivery platform. *Advanced Drug Delivery Reviews* 64, 836–851. <https://doi.org/10.1016/j.addr.2012.02.002>
- García, M.C., Cuggino, J.C., 2018. Stimulus-responsive nanogels for drug delivery, in: *Stimuli Responsive Polymeric Nanocarriers for Drug Delivery Applications*, Volume 1. Elsevier, pp. 321–341. <https://doi.org/10.1016/B978-0-08-101997-9.00016-3>
- Gruber, A., Işık, D., Fontanezi, B.B., Böttcher, C., Schäfer-Korting, M., Klinger, D., 2018. A versatile synthetic platform for amphiphilic nanogels with tunable hydrophobicity. *Polymer Chemistry* 9, 5572–5584. <https://doi.org/10.1039/C8PY01123K>
- Hasegawa, U., Nomura, S.M., Kaul, S.C., Hirano, T., Akiyoshi, K., 2005. Nanogel-quantum dot hybrid nanoparticles for live cell imaging. *Biochemical and Biophysical Research Communications* 331, 917–921. <https://doi.org/10.1016/j.bbrc.2005.03.228>
- Hayashi, C., Hasegawa, U., Saita, Y., Hemmi, H., Hayata, T., Nakashima, K., Ezura, Y., Amagasa, T., Akiyoshi, K., Noda, M., 2009. Osteoblastic bone formation is induced by using nanogel-crosslinking hydrogel as novel scaffold for bone growth factor. *Journal of Cellular Physiology* 220, 1–7. <https://doi.org/10.1002/jcp.21760>

- Khan, I.U., Serra, C.A., Anton, N., Vandamme, T., 2013. Continuous-flow encapsulation of ketoprofen in copolymer microbeads via co-axial microfluidic device: Influence of operating and material parameters on drug carrier properties. *International Journal of Pharmaceutics* 441, 809–817. <https://doi.org/10.1016/j.ijpharm.2012.12.024>
- McAllister, K., Sazani, P., Adam, M., Cho, M.J., Rubinstein, M., Samulski, R.J., DeSimone, J.M., 2002. Polymeric Nanogels Produced via Inverse Microemulsion Polymerization as Potential Gene and Antisense Delivery Agents. *J. Am. Chem. Soc.* 124, 15198–15207. <https://doi.org/10.1021/ja027759q>
- Miller, C.A., 1988. Spontaneous Emulsification Produced by Diffusion — A Review. *Colloids and Surfaces* 29, 89–102. [https://doi.org/10.1016/0166-6622\(88\)80173-2](https://doi.org/10.1016/0166-6622(88)80173-2)
- Molina-García, L., Santos, J.L.M., Ruiz-Medina, A., Llorent-Martínez, E.J., 2013. Determination of ketoprofen based on its quenching effect in the fluorescence of quantum dots. *Journal of Food and Drug Analysis* 21, 426–431. <https://doi.org/10.1016/j.jfda.2013.09.007>
- Oishi, M., Sumitani, S., Nagasaki, Y., 2007. On–Off Regulation of ¹⁹F Magnetic Resonance Signals Based on pH-Sensitive PEGylated Nanogels for Potential Tumor-Specific Smart ¹⁹F MRI Probes. *Bioconjugate Chemistry* 18, 1379–1382. <https://doi.org/10.1021/bc7002154>
- O’Reilly, R.K., Hawker, C.J., Wooley, K.L., 2006. Cross-linked block copolymer micelles: functional nanostructures of great potential and versatility. *Chemical Society Reviews* 35, 1068. <https://doi.org/10.1039/b514858h>
- Peng, H., Stolwijk, J.A., Sun, L.-N., Wegener, J., Wolfbeis, O.S., 2010. A Nanogel for Ratiometric Fluorescent Sensing of Intracellular pH Values. *Angewandte Chemie International Edition* 49, 4246–4249. <https://doi.org/10.1002/anie.200906926>
- Qiao, Z.-Y., Zhang, R., Du, F.-S., Liang, D.-H., Li, Z.-C., 2011. Multi-responsive nanogels containing motifs of ortho ester, oligo(ethylene glycol) and disulfide linkage as carriers of hydrophobic anti-cancer drugs. *Journal of Controlled Release* 152, 57–66. <https://doi.org/10.1016/j.jconrel.2011.02.029>
- Raemdonck, K., Demeester, J., De Smedt, S., 2009. Advanced nanogel engineering for drug delivery. *Soft Matter* 5, 707–715. <https://doi.org/10.1039/B811923F>
- Rehman, A.U., Collot, M., Klymchenko, A.S., Akram, S., Mustafa, B., Vandamme, T., Anton, N., 2019. Spontaneous nano-emulsification with tailor-made amphiphilic polymers and related monomers. *European Journal of Pharmaceutical Research* 1, 27–36. [https://doi.org/10.34154/2019-EJPR.01\(01\).pp-27-36/eurass](https://doi.org/10.34154/2019-EJPR.01(01).pp-27-36/eurass)
- Ryu, J.-H., Chacko, R.T., Jiwanich, S., Bickerton, S., Babu, R.P., Thayumanavan, S., 2010a. Self-Cross-Linked Polymer Nanogels: A Versatile Nanoscopic Drug Delivery Platform. *Journal of the American Chemical Society* 132, 17227–17235. <https://doi.org/10.1021/ja1069932>

- Ryu, J.-H., Jiwanpanich, S., Chacko, R., Bickerton, S., Thayumanavan, S., 2010b. Surface-Functionalizable Polymer Nanogels with Facile Hydrophobic Guest Encapsulation Capabilities. *Journal of the American Chemical Society* 132, 8246–8247. <https://doi.org/10.1021/ja102316a>
- Sasaki, Y., Akiyoshi, K., 2010. Nanogel engineering for new nanobiomaterials: from chaperoning engineering to biomedical applications. *The Chemical Record* n/a-n/a. <https://doi.org/10.1002/tcr.201000008>
- Torchilin, V.P., 2005. Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery* 4, 145–160. <https://doi.org/10.1038/nrd1632>
- Wang, X., Anton, N., Ashokkumar, P., Anton, H., Fam, T.K., Vandamme, T., Klymchenko, A.S., Collot, M., 2019. Optimizing the Fluorescence Properties of Nanoemulsions for Single Particle Tracking in Live Cells. *ACS Applied Materials & Interfaces* 11, 13079–13090. <https://doi.org/10.1021/acsami.8b22297>
- Yan, S., Sun, Y., Chen, A., Liu, L., Zhang, K., Li, G., Duan, Y., Yin, J., 2017. Templated fabrication of pH-responsive poly(L-glutamic acid) based nanogels via surface-grafting and macromolecular crosslinking. *RSC Advances* 7, 14888–14901. <https://doi.org/10.1039/C7RA00631D>
- Yu, M., Yuan, W., Li, D., Schwendeman, A., Schwendeman, S.P., 2019. Predicting drug release kinetics from nanocarriers inside dialysis bags. *J. Control. Release* 315, 23–30. <https://doi.org/10.1016/j.jconrel.2019.09.016>
- Zhuang, J., Jiwanpanich, S., Deepak, V.D., Thayumanavan, S., 2012. Facile Preparation of Nanogels Using Activated Ester Containing Polymers. *ACS Macro Letters* 1, 175–179. <https://doi.org/10.1021/mz200123f>