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***In vitro* study of versatile drug formulations based on α -cyclodextrin and polyethylene glycol using colloidal tectonics**

Loïc Leclercq,^{1,*} Jean-François Dechézelles,¹ Gaétan Rauwel,² and Véronique Nardello-Rataj¹

¹ Univ. Lille, CNRS, ENSCL, UMR 8181 - UCCS - Unité de Catalyse et Chimie du Solide, F-59 000 Lille, France.

Email: loic.leclercq@univ-lille.fr

² Laboratoires Anios, Pavé du Moulin, F-59 260 Lille-Hellemmes, France.

ABSTRACT: In response to the environmental problems, we propose herein to use a common base of formulation leading to a set of galenic systems such as ethanol-free hydrogels, surfactant-free and silica-free Pickering emulsions and microcapsules. All of the systems are developed from the self-assembly of α -cyclodextrin (α -CD) and polyethylene glycol (PEG) using the colloidal tectonics approach. Firstly, α -CD and PEG were mixed to obtain stable hydrogels **loaded** with antifungal drugs in the form of complexes with 2-hydroxypropyl- β -CD to increase the drugs solubility in the gels. Secondly, oil-in-water Pickering emulsions were prepared from the **unloaded** hydrogels by adding paraffin oil containing antifungals in their native forms. Thirdly, these emulsions were dried to obtain **loaded** microcapsules. **The different systems were characterized by means of physicochemical measurements (microscopy, pH, rheology and stability) and anti-pathogen activities.** Their properties are close to those of commercially available ones but superior for their stability, with even a clear enhancement of the anti-pathogen activity (up to 1.6). All formulations reduce the potential drawbacks of the commonly used excipients and are in line with the current trend of simplifying formulas in terms of ingredients.

KEYWORDS: Colloidal tectonics, Drug delivery systems, Hydrogels, Pickering emulsions, Cyclodextrinosomes, Antifungal drug

INTRODUCTION

To treat skin and mucous membranes infections, imidazole drugs (e.g. miconazole, MC, or econazole, EC, Figure 1), which have a broad spectrum activity against fungus and Gram-positive bacteria, are commonly used [1]. As MC and EC are very poorly water-soluble (6.5×10^{-5} and 2.9×10^{-8} M), their nitrate salts (MCN and ECN) are more commonly used (3.7×10^{-4} and 1.6×10^{-3} M) [2]. For instance, Micatin® and Pevaryl® are paraffin oil-in-water emulsions stabilized by macrogol derivatives (e.g. peglicol 5 oleate and pegoxol 7 stearate) and loaded with 2% of MCN and 1% of ECN, respectively. Otherwise, MC (2%) can also be used in the form of hydrogel (Daktarin®) stabilized by pregelatinized potato starch in combination with polysorbate 20, glycerol and ethanol as emulsifiers and solubilizers. Although the macrogol derivatives are considered as not harmful, some allergic reactions have been reported [3,4].

In this context, Pickering emulsions (i.e. stabilized by nanoparticles such as silica) are an elegant way to avoid molecular emulsifiers [5]. However, their human health risks are not totally clear [6] as silica nanoparticles are able to penetrate the *stratum corneum* [7]. A smart alternative is to exploit self-assembled nanoparticle structures according to the colloidal tectonics concept allowing the construction of colloidal systems from molecular tectons [8,9]. For instance, cyclodextrins (CDs) are able to emulsify oil and water mixtures due to the formation of insoluble oil/CD inclusion complexes (ICs) [10] inducing the emergence of nanoparticle-like structures made of aggregated ICs able to stabilize Pickering emulsions [11,12]. Unlike silica nanoparticles, these assemblies are easily dissociated without harmful side effects [13]. Finally, Pickering emulsion droplets are suitable templates to obtain microcapsules (cyclodextrinosomes) with potential applications in pharmacy [14].

We propose herein to use low toxicity tectons such as native α -CD and polyethylene glycol (PEG) to formulate easily three systems (ethanol-free hydrogels, surfactant-free and silica-free Pickering emulsions and microcapsules) able to deliver MC, MCN and ECN to treat skin infections. **The preparation, the physicochemical and the *in vitro* biocidal properties of these systems are reported and compared to commercial formulations.**

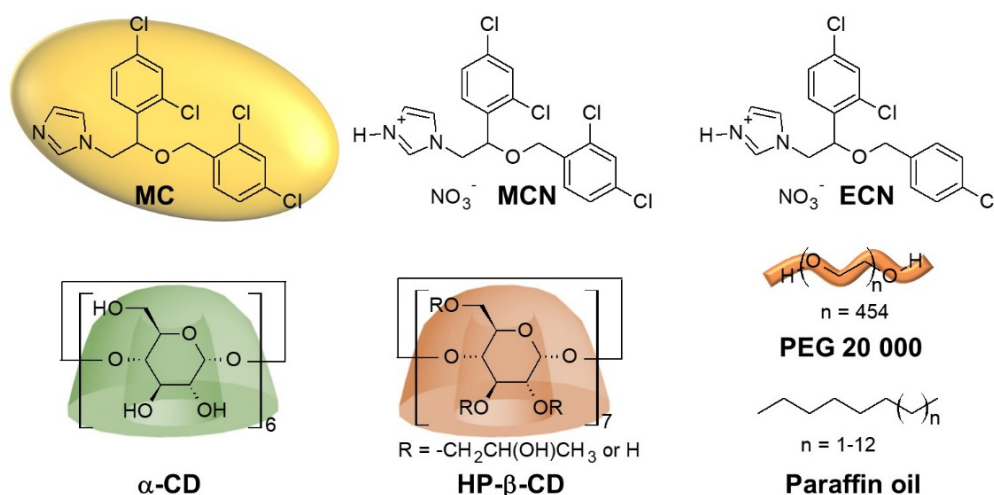


Figure 1. Antifungal agents (miconazole, MC, miconazole nitrate, MCN, or econazole nitrate, ECN), cyclodextrins (α -CD and 2-hydroxypropyl- β -CD, HP- β -CD), polyethylene glycol (PEG 20 000) and paraffin oil used in this work.

MATERIALS AND METHODS

General Information

All chemicals, Petri dishes and sterile disks were purchased from Sigma-Aldrich and Fluka. Sterile water for injection was used in all experiments (Fresenius-Kabi, France). All mixing, dispersing and emulsification were performed using Ultra-turrax (T10 basic, IKA, Germany).

Phase solubility diagram

Increasing concentrations of HP- β -CD (0-0.23 M) were added to excess amounts of MCN or ECN. Suspensions were stirred at 25 °C during 10 days until reaching the equilibrium. All suspensions were filtered through 0.45 μ m membrane filter (Millipore), suitably diluted with ethanol and spectrophotometrically analyzed (Cary 50 Probe Varian) at 272 nm [15].

PEG/ α -CD hydrogels and Pickering emulsions

To prepare 100 g of PEG/ α -CD hydrogel, PEG 20 000 (2.9 g) was weighed and powdered in a mortar before adding water (80.9 g). The mixture was sonicated and heated at 80 °C under stirring for 10 min before adding α -CD (16.2 g). The mixture was kept at 80 °C under stirring for 25 min and was then thoroughly mixed (11 500 rpm, 90 s). Finally, the solution was cooled overnight at 3 °C to give the hydrogel. Typical emulsion was prepared from the previous PEG/ α -CD hydrogel by adding 50 wt.% of paraffin oil. The emulsification was performed at 11 500 rpm (180 s, 25 °C).

PEG/ α -CD hydrogels loaded with MC, MCN or ECN.

To prepare 10 g of topical PEG/ α -CD hydrogel loaded with 1% of MC, MCN or ECN, the drug (0.1 g) and HP- β -CD (1.35 g) were weighed. A homogeneous paste of HP- β -CD was prepared in a mortar by adding ethanol/water mixture (1:1) in small quantities. Drug powder was then added to this paste in portions, with continuous kneading, for 3 hours. An appropriate quantity of ethanol/water mixture (1:1) was added further to maintain suitable consistency of the paste. This paste was dried at 45 °C for 24 hours. The drug/HP- β -CD ICs was powdered and stored in a sealed container. The PEG/ α -CD hydrogel (8.55 g) in the gel phase was heated at 80 °C for 30 min to give the PEG/ α -CD hydrogel in the sol phase. Drug/HP- β -CD ICs were added and thoroughly mixed (11 500 rpm, 180 s). Finally, the solution was cooled overnight at 3 °C to give the hydrogel. The topical PEG/ α -CD hydrogel (2%) was prepared from drug (0.2 g) and HP- β -CD (2.82 g) as described previously.

PEG/ α -CD Pickering emulsions loaded with MC, MCN or ECN

To prepare 10 g of topical PEG/ α -CD emulsion loaded with 1% of MC, MCN or ECN, the unloaded PEG/ α -CD hydrogel (4.933 g) was heated to obtain the sol phase. Benzoic acid (0.02 g) was added and thoroughly mixed (11 500 rpm, 180 s). Finally, the obtained hydrogel in the sol phase was cooled to room temperature for 30 min and let overnight at 3 °C to give the PEG/ α -CD hydrogel in the gel phase. Drug (0.1 g) was dissolved in paraffin oil

(4.947 g) and added to the hydrogel. The emulsification was performed at 11 500 rpm (180 s, 25 °C). The topical PEG/ α -CD emulsion (2%) was prepared from PEG/ α -CD hydrogel (4.883 g), benzoic acid (0.02 g), drug (0.2 g) and paraffin oil (4.897 g) as described previously.

Microscopy study

Optical microphotographs of the samples were obtained using a light microscope (Standard 25 ICS, Zeiss) coupled with a Charge-Coupled-Device Camera (Digital Still Camera, SONY). For emulsions, optical microphotographs were analyzed with ImageJ software (National Institutes of Health, USA) to obtain the droplets size distribution. The distribution function was obtained by treatment of at least 250 individual measurements of droplet diameters in the microphotographs with log-normal function using OriginPro 8[®], USA. The median droplet diameter (d_m) was determined in triplicate. Scanning electron microscopy (SEM) observations were performed with a Hitachi FlexSem 1000 operating at 25 kV and at a vacuum pressure of 30 Pa. *In situ* SEM observations of the emulsion and hydrogel were carried out with WETSEM QX-102 capsules (Quantomix Ltd). WETSEM capsules allow performing SEM characterizations without drying the sample or removing the water phase. We proceed as following for the sample preparation. 15 μ L of sample were deposited in the liquid dish of the capsule and sealed with the stub. We focused on the metal support grid with the SE detector before changing to the BSE detector to image the sample. SEM observations of emulsions were performed by depositing droplets onto a carbon conductive adhesive tape stuck on a stub after air-drying.

Physicochemical investigations of hydrogels and emulsions

The prepared hydrogels or emulsions were inspected visually for their color and homogeneity. In addition, the type of topical emulsions was determined by electric conductivity measurement. Emulsion type was also inferred by observing what happened when a drop of each emulsion was added to a volume of oil or water. The pH of the prepared hydrogels or emulsions was measured using a digital pH meter (pH 330i/SET, WTW, Germany). 1 g of the sample was stirred in distilled water (100 mL). Then, pH measurement was performed on the 1% solution. The test was performed in triplicate and the mean was calculated. Rheological properties of emulsions were evaluated using a Malvern Kinexus rheometer (Malvern Instrument) equipped with parallel plate geometry under controlled temperature. The diameter of the plates was 20 mm and the gap was 1 mm. The temperature was controlled using a Peltier temperature control device located below the lower plate with an accuracy of 0.01 °C. Stress-controlled measurements were performed imposing a logarithmic stress ramp increase followed by a reverse stress decrease from 0 to 500 Pa.s (0 to 3 500 Pa.s for Daktarin[®]). The stability of hydrogels or emulsions was evaluated at constant temperature (37 °C) by multiple light scattering using a Turbiscan Lab Expert (Formulaction). The samples were placed in cylindrical glass tubes and subjected to Turbiscan Lab Expert stability analysis. The analysis of stability was carried out as a variation of backscattering (Δ BS) profiles. Measurements were carried out using a pulsed near-IR light-emitting diode (LED) at a wavelength of 880 nm. Two different synchronous optical sensors

received the light transmitted through and backscattered by samples at an angle of 180° and 45° with respect to the incident radiation, respectively. The two sensors scanned the entire height of the sample for 7 days.

Antimicrobial and antifungal tests using disk diffusion method

The biocidal activity against *Staphylococcus aureus* and *Candida albicans* was evaluated using the Kirby-Bauer test (disk diffusion assay) for all prepared hydrogels or emulsions with or without antifungal drugs (1 or 2%) and compared to the commercially available formulations, *i.e.* Micatin®, Pevaryl®, and Daktarin®. The Kirby-Bauer method relies on the inhibition of bacterial or fungal growth measured under standard conditions. For this purpose, Petri dishes (90 mm) containing a culture agar medium were uniformly and aseptically inoculated with *S. aureus* (CIP 4.83) or *C. albicans* (DSM 1386). More specifically, Tryptone Soya Agar (TSA: tryptone (15.0 g), soytone (5.0 g), sodium chloride (5.0 g), agar (15.0 g), water *q.s.* 1 L) and Malt Extract Agar (MEA: malt extract (30.0 g), agar (15.0 g), water *q.s.* 1 L) were used for the culture of *S. aureus* and *C. albicans*, respectively. Then, sterile disks (obtained from Fluka) impregnated with the various prepared formulations, were placed at the center of the agar plates (*S. aureus*: 48 hours at 37 ± 1 °C and *C. albicans*: 48 hours at 30 ± 1 °C). The organisms were grown on the agar plate while the drug diffuse and inhibit the microorganism growth. This study was performed in triplicate for each formulation. The pH of the culture media was 5.6 ± 0.2 and 7.3 ± 0.2 for *C. albicans* and *S. aureus*, respectively.

Loaded microcapsules

The loaded emulsion was air-dried at 25 °C during 24 hours (with or without additional vacuum treatment) in order to remove the water continuous phase (or the oil-core).

Antimicrobial and antifungal tests of microcapsules

The air-dried (25 °C during 24 hours, without additional vacuum treatment) loaded microcapsules were slightly compressed into tablets (total weight: 200 mg, 1 cm of diameter) without any coating. The tablet was loaded at the center of the agar plates infected with *S. aureus* (CIP 4.83) or *C. albicans* (DSM 1386) and was moistened with 80 µL of water. The infected agar plates were incubated for 48h at 37 ± 1 °C for *S. aureus* or 30 ± 1 °C for *C. albicans*. The area of inhibition for each formulation was then measured. This study was performed in triplicate.

RESULTS

Preliminary investigations

(i) *Preparation of PEG/α-CD hydrogels.* Typical hydrogels can be prepared from aqueous solutions of α-CD and PEG 20 000. The α-CD weight is kept constant (16.2 g), whereas the PEG amount is varied to yield a series of hydrogels (0.4-2.9 g, water *q.s.* 100 g). Their stability is examined as a function of storage time at 25 °C (Figure 2). The hydrogels stability, after 2 months, is clearly higher with 2.9 g of PEG than with 0.4 g. For this stable hydrogel (α-CD 16.2 g, PEG 2.9 g, water *q.s.* 100 g), a sol-gel transition temperature of *ca.* 68 °C is measured.



Figure 2. Photographs of various hydrogels prepared from aqueous solutions of α -CD and PEG 20 000 at $t = 0$ (top) and 2 months (bottom, H = hydrogel phase and W = water phase).

The presence of numerous crystallites has been confirmed by *in situ* observations of the gel using optical (with or without polarized light) and wet scanning electron microscopy (WETSEM) (Figure 3A, A' and B).

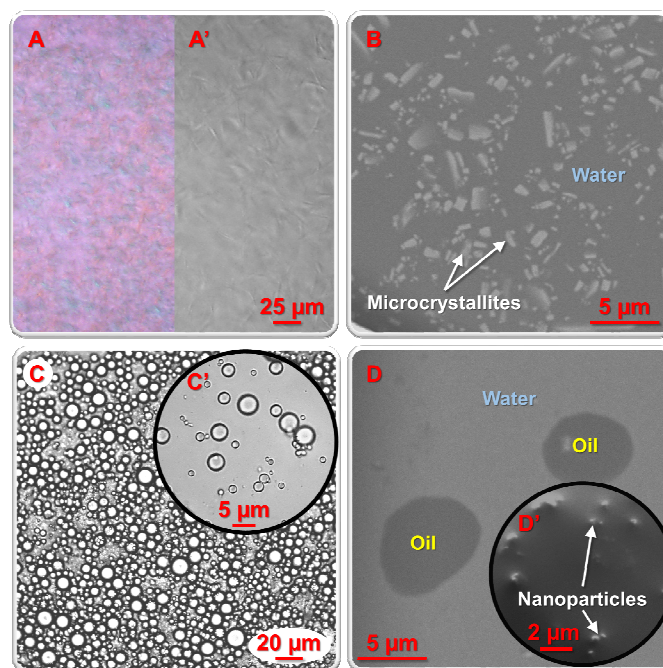


Figure 3. Observations of PEG/ α -CD systems in the form of hydrogel (α -CD 16.2 g, PEG 2.9 g, water *q.s.* 100 g) using optical (A), polarized (A') and wet scanning electron microscopy, WETSEM, (B) and of Pickering emulsion (α -CD 8.1 g, PEG 1.45 g, water 40.45 g, paraffin oil *q.s.* 100 g) using optical (C and C'), WETSEM (D) and SEM microscopy (D').

(ii) *Preparation of PEG/ α -CD emulsions.* The PEG/ α -CD hydrogels can be used to prepare Pickering emulsions by addition of an organic phase. Indeed, typical emulsions can be readily obtained from the previous hydrogel by adding 50 wt.% of paraffin oil. After emulsification (11 500 rpm, 180s), the oil/water biphasic system provides whitish and stable emulsions. The electrical conductivity of the emulsions indicates a water continuous phase. The oil-in-water emulsion structure was investigated using optical light and WETSEM microscopy (Figure 3C, C' and D).

These photographs show that no crystallite is observed in the continuous aqueous phase or onto the droplet surface. However, we can observe the presence of nanoparticle-like structures (< 500 nm) by SEM in dry condition (see Figure 3D').

(iii) *Aqueous solubility of azole drugs.* If emulsions can easily be used to deliver hydrophobic antifungal drugs (MC, MCN and ECN) due to their high solubility in the oil phase, their direct solvation in the hydrogels is very limited (see above). To solve this drawback, the highly water-soluble 2-hydroxypropyl- β -CD (HP- β -CD) is a good alternative to enhance the water-solubility by complexation and to avoid the use of ethanol as in commercial hydrogel (Daktarin®). Therefore, the phase solubility diagrams of azole drugs in unbuffered aqueous HP- β -CD solution have been investigated (Figure 4).

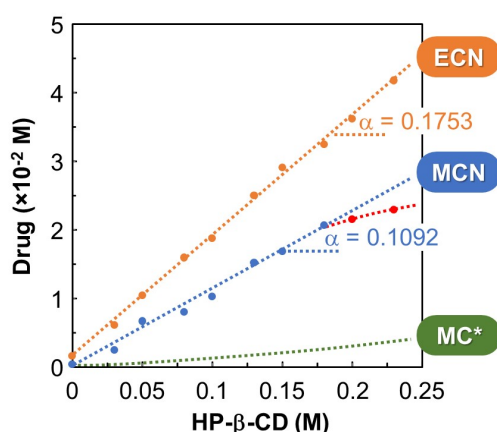


Figure 4. Aqueous phase solubility diagrams of drug (MC, MCN or ECN) with HP- β -CD at 25 °C in unbuffered condition (* Taken from ref. 16).

From these curves and the method of Higuchi and Connors [15], the apparent binding constants were calculated and compared with the data available in the literature (Table 1) [16].

Table 1. Intrinsic solubility of MC, MCN and ECN and binding constants (K_1 and K_2) obtained for drugs/HP- β -CD inclusion complexes.

	MC ^a	MCN ^b	ECN ^b
Intrinsic solubility (M)	6.5×10^{-5}	4.0×10^{-4} (3.7×10^{-4}) ^c	1.6×10^{-3}
K_1 (M ⁻¹)	127	306 (280) ^c	133
K_2 (M ⁻¹)	2.20	-	-

^a Taken from ref. 16. ^b This work. ^c Taken from ref. 2.

Physicochemical investigations of hydrogels and emulsions

(i) *Investigated formulations.* All the investigated compositions are presented in Table 2. In addition, the commercial emulsions (Pevaryl® and Micatin®) and gels (e.g. Daktarin®) have also been investigated for reference purposes (positive controls). In contrast, the unloaded hydrogels or emulsions are used as negative controls. It is noteworthy that benzoic acid has been added as preservative to prevent the growth of bacteria, fungi and yeasts in the aqueous phase of the emulsions [1]. In contrast, for hydrogels, as the azole drugs are antifungal agents that

also has antibacterial activity and as the azoles are solubilized in the aqueous phase by the formation of ICs with the HP- β -CD, the addition of preservatives is not required.

Table 2. Final composition of hydrogels (H) and Pickering emulsions (E).^a

		Quantity (g)						
Hydrogels		H	H MC 1	H MC 2	H MCN 1	H MCN 2	H ECN 1	H ECN 2
Ingredients	MC	-	1	2	-	-	-	-
	MCN	-	-	-	1	2	-	-
	ECN	-	-	-	-	-	1	2
	α -CD	13.8	13.8	11.3	13.8	11.3	13.8	11.3
	PEG ^b	2.5	2.5	2.0	2.5	2.0	2.5	2.0
	HP- β -CD	13.5	13.5	28.2	13.5	28.2	13.5	28.2
	Water	70.2	69.2	56.5	69.2	56.5	69.2	56.5
Emulsions		E	E MC 1	E MC 2	E MCN 1	E MCN 2	E ECN 1	E ECN 2
Ingredients	MC	-	1	2	-	-	-	-
	MCN	-	-	-	1	2	-	-
	ECN	-	-	-	-	-	1	2
	α -CD	8.0	8.0	7.9	8.0	7.9	8.0	7.9
	PEG ^b	1.43	1.43	1.43	1.43	1.43	1.43	1.43
	Benzoic acid	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Water	39.9	39.9	39.5	39.9	39.5	39.9	39.5
Paraffin	50.47	49.47	48.97	49.47	48.97	49.47	48.97	

^a For details on the preparation: see the experimental section. ^b PEG 20000.

(ii) *Texture and pH.* All the obtained hydrogels or oil-in-water Pickering emulsions are white as the commercial samples. More specifically, our formulated hydrogels present non-greasy paste texture whereas the Daktarin[®] is sticky and the emulsions present cream texture except Pevaryl[®] which provides a fluid texture (Table 3). The pH of all our formulations is between 3.8 and 7.0, which corresponds to the normal pH range of the skin or mucous membranes (pH 4.0 to 7.0). It should be noted that the pH of the commercial samples is very similar to that of our formulations.

Table 3. Texture, pH, and median droplet diameter, d_m , of Pickering emulsions (E) and hydrogels (H) compared to commercial samples (PEVARYL[®], MICATIN[®] and DAKTARIN[®]).^a

Hydrogel	Texture ^b	pH ^c	Emulsion	Texture ^b	pH ^c	d_m (μ m) ^d
H	Non-greasy paste	7.0	E	Cream	6.1	4.5
DAKTARIN [®]	Sticky paste	5.8	E MC 1	Cream	5.9	3.7
H MC 1	Non-greasy paste	5.9	E MC 2	Cream	5.8	3.4
H MC 2	Non-greasy paste	6.1	MICATIN [®]	Cream	4.0	5.3
H MCN 1	Non-greasy paste	4.5	E MCN 1	Cream	3.9	6.9
H MCN 2	Non-greasy paste	4.3	E MCN 2	Cream	3.8	4.5
H ECN 1	Non-greasy paste	4.7	PEVARYL [®]	Fluid	3.9	10.1
H ECN 2	Non-greasy paste	4.4	E ECN 1	Cream	4.2	3.6
			E ECN 2	Cream	4.0	4.4

^a For compositions, see Table 2. ^b Visual texture of the Pickering emulsions or hydrogels. ^c Determined after dilution of the concentrated emulsions (1 wt. % in water). ^d Obtained after dilution in water using OriginPro 8[®], (USA) with log-normal fitting, S.D. = 10%.

(iii) *Microstructures*. The microstructures of the hydrogel and Pickering emulsions have been examined by optical microscopy. The images obtained for the **loaded** hydrogels are very similar to that of the **unloaded** hydrogels and confirm the presence of packed PEG/ α -CD polypseudorotaxane domains in the mixture (see above). For emulsions, a statistical analysis of the images was used to determine the median droplet diameter (see d_m in Table 3). All emulsions behave similarly, giving a monomodal size distribution with a slight polydispersity. The average droplet sizes, comprised between 3.4 and 6.9 μm , are also in the same order of magnitude of the commercial references (5.3 and 10.1 μm for Micatin[®] and Pevaryl[®], respectively).

(iv) *Rheology*. Next, we performed a rheological comparison between the commercial samples and our formulations. For shake of clarity, only hydrogels and emulsions formulated with ECN (1 and 2%) as well as the commercial ones are presented in Figure 5.

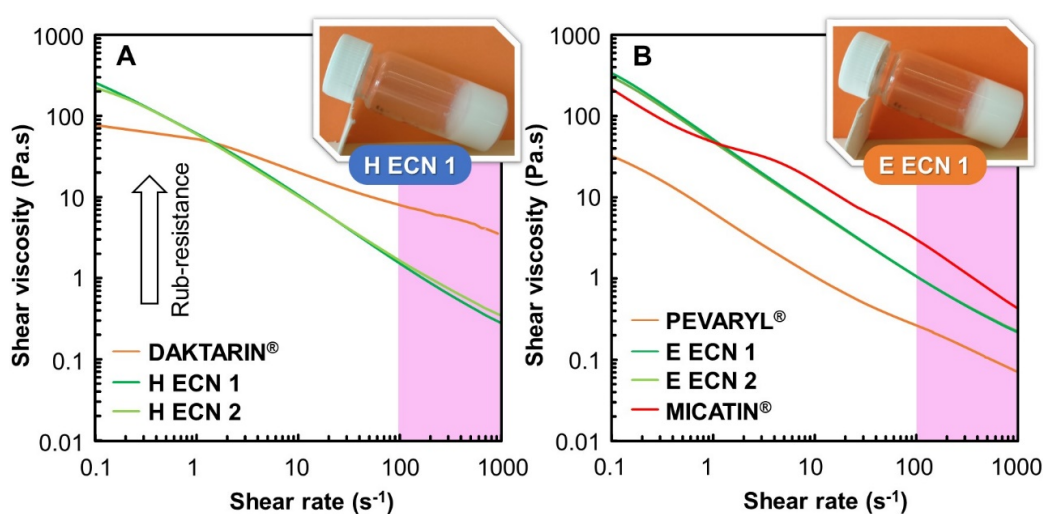


Figure 5. Shear viscosity as a function of shear rate of some hydrogels (A) and emulsions (B) as well as for commercial samples at 25 °C (for compositions, see Table 2). The pink area indicates the regime when the emulsion is spread on the skin.

All prepared formulations have similar rheological behavior whatever the active ingredient. This observation is indicative of the absence of interactions between the antifungal drugs and the α -CD. In addition, the prepared formulations are highly viscous in storage conditions but exhibit low viscosity when applied onto the skin (*i.e.* under shear rate). The viscosity recorded in the high-shear regime (from 100 to 1000 s⁻¹) can be used to predict the spreadability of creams on the skin. In this range, as the viscosity of the hydrogels and emulsions remain inferior to 2 Pa.s, they are clearly suitable for topical applications on skin. More specifically, the viscosities of emulsions are comprised between the two commercial references (Micatin[®] and Pevaryl[®]) whereas the rub-resistance of Daktarin[®] is greater than our hydrogels: the prepared hydrogels viscosity is reduced up to a factor 4.5 between 100 to 1000 s⁻¹. Therefore, their spreadability is better than the commercial reference (Daktarin[®]). However, as hydrogels can also be used to treat fungal infections of the mouth, throat and gut, the viscosity at low shear rate (< 1 s⁻¹) is essential to avoid the obstruction of the throat by the gel. Daktarin[®] and prepared hydrogels exhibit the

same viscosity at a certain shear rate ($\approx 1.6 \text{ s}^{-1}$) but behave differently when applied: the viscosity of prepared hydrogels are higher at low shear rates while the viscosity of Daktarin[®] is higher at high shear rates. However, when the external force is removed, Daktarin[®] and prepared hydrogels exhibit different rearrangement in order to restore the initial network structure over a period of time. For Daktarin[®], the viscosity as a function of decreasing shear rate is superimposed on the viscosity as a function of increasing shear rate due to a fast structure recovery: non-thixotropic behavior (not shown in Figure 5). In contrast, for the prepared hydrogels, the time taken to restore the structure are higher and therefore the “down curves” are below the “up curves”: thixotropic behavior (not shown in Figure 5). Even, if the viscosities of formulated hydrogels are higher than those of the commercial one, their thixotropic as well as the water-miscible properties are suitable for use as oral gel with a reduction of choking hazard.

(v) *Stability*. To quantify the emulsion stability, multiple light scattering experiments have been performed for 7 days at 37 °C (normal body temperatures). For shake of clarity, only hydrogel and emulsion formulated with ECN (1%) are presented in Figure 6. It is noteworthy that the behaviors observed for the other drugs are perfectly similar. The increase of backscattered light in absolute value (ΔBS) at mid-height of the sample indicates a slight increase in size due to either a rearrangement of water domains in the hydrogels or a slow limited-coalescence process for Pickering emulsions. However, the kinetics of these effects is very minor over 7 days ($\Delta\text{BS} < 2\%$ in absolute value). Moreover, after 12 months, only a small upper water phase corresponding to 0.5% of the initial total volume is observed for hydrogels whereas the Pickering emulsions exhibit a very long-term stability due to the limited-coalescence process which cannot be considered as a true instability.

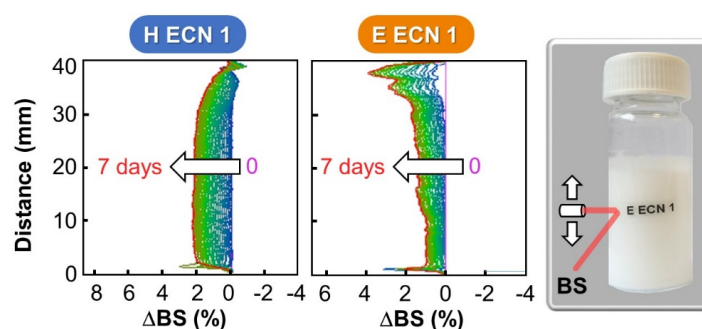


Figure 6. Evolution of backscattering (ΔBS) versus sample height and time for typical hydrogel and emulsion at 37 °C (for compositions, see Table 2). The inset shows the principle of multiple light scattering experiments.

Antifungal and antimicrobial activity of hydrogels and emulsions

(i) *Antifungal activity*. Disk diffusion tests have been performed to evaluate the efficacy of our hydrogels and emulsions against *C. albicans*. These results are compared to positive control (Micatin[®], Pevaryl[®] and Daktarin[®]) and negative controls (unloaded hydrogels or emulsions). As depicted in Figures 7, and 8, the contact biocidal property can be measured by the zone of inhibition around the impregnated disk after 48 h incubation. The negative control experiments give no zone of inhibition whatever the drug delivery platform. The diameters of the

zone of inhibition for hydrogels loaded with MC, MCN and ECN against *C. albicans* are very similar to the commercial formulation (Daktarin®, Figure 7).

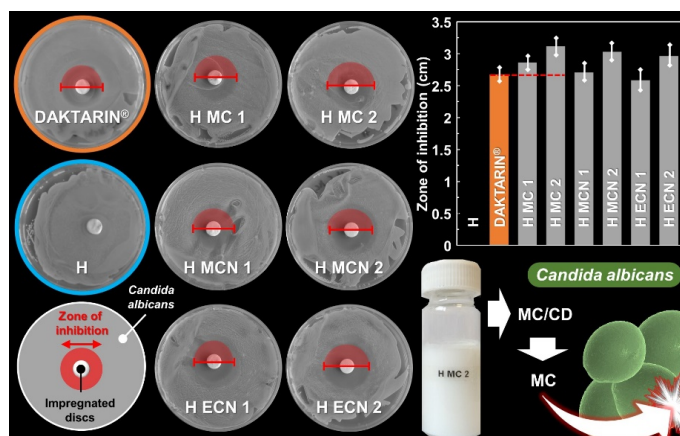


Figure 7. Typical photographs of the zone of inhibition obtained by the Kirby-Bauer method against *C. albicans* of the prepared hydrogels (for compositions, see Table 2) compared to Daktarin®. The top inset shows the average zone of inhibition obtained after triplicate.

This result indicates that these hydrogels are at least as effective in terms of contact biocidal property as Daktarin® whatever the amount of active ingredient present. These good activities are not related to viscosity. Indeed, the prepared hydrogels are more viscous than Daktarin® reference but their biocidal activities are quite similar due to the presence of HP-β-CD. Indeed, the formation of drug/HP-β-CD ICs improves the efficacy and bioavailability of these drugs whatever the molecular structure and the physicochemical properties of the drug.

In contrast, for the Pickering emulsions, the biocidal activities against *C. albicans* depend on the structure and the properties of the drugs. Indeed, as depicted in Figure 8, the antifungal activity increases in the order $E MC x \approx E ECN x < E MCN x$ (with $x = 1$ or 2).

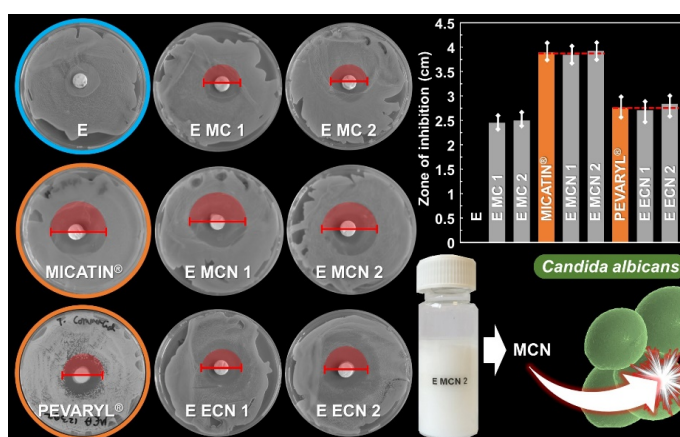


Figure 8. Typical photographs of the zone of inhibition obtained by the Kirby-Bauer method against *C. albicans* of the prepared emulsions (for compositions, see Table 2) compared to Micatin® and Pevaryl®. The top inset shows the average zone of inhibition obtained after triplicate.

However, the zone of inhibition after 48 h incubation for emulsions loaded with MCN is very similar to that of Micatin®. Similar behavior is observed for emulsions loaded with ECN and Pevaryl®. The weak antifungal activity of

emulsions **loaded** with MC can be related to the state of ionization of the drug. Indeed, ionized species are able to penetrate more readily into biological membranes.

(ii) *Antimicrobial activity.* A typical Gram-positive bacterium (*S. aureus*) was also chosen for the disk diffusion tests because MC, MCN and ECN are also active *in vitro* against it. The results are shown in Figures 9 and 10. The diameters of the zone of inhibition for hydrogels **loaded** with MC, MCN and ECN are clearly improved compared to Daktarin® (up to a factor of 1.6, Figure 9). As previously mentioned, this behavior is due to the formation of ICs between the drug and the HP- β -CD leading to better bioavailability of the drug. Despite the fact that the MCN presents better antibacterial activities than MC, the **unloaded** form is preferred for oral application since the pH of the mouth has to be kept between 6.2 and 7.4.

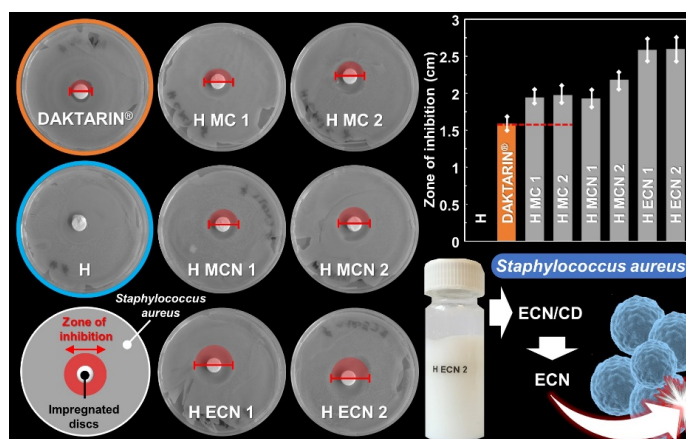


Figure 9. Typical photographs of the zone of inhibition obtained by the Kirby-Bauer method against *S. aureus* of the prepared hydrogels (for compositions, see Table 2) compared to Daktarin®. The top inset shows the average zone of inhibition obtained after triplicate.

For the Pickering emulsions, the antibacterial activity increases in the order $E\ MC\ x \approx E\ MCN\ 1 < E\ MCN\ 2 < E\ MCN\ x$ (with $x = 1$ or 2 , see Figure 10).

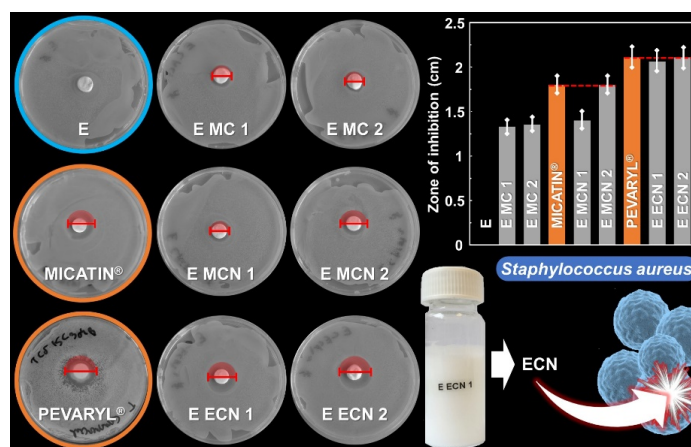


Figure 10. Typical photographs of the zone of inhibition obtained by the Kirby-Bauer method against *S. aureus* of the prepared emulsions (for compositions, see Table 2) compared to Micatin® and Pevaryl®. The top inset shows the average zone of inhibition obtained after triplicate.

However, the zone of inhibition, after 48 h incubation for emulsions loaded similar amounts of drug, is very close to that of the commercial references (Micatin® and Pevaryl®). As previously mentioned, the weak antibacterial activity observed for emulsions loaded with MC can be related to the state of weak ionization of MC leading to less ability to penetrate membranes.

Alternative formulations based on microcapsules

(i) *Preparation and characterization.* Finally, as the cyclodextrinosomes (i.e. microcapsules), described previously by Mathapa and Paunov, could find applications as drug delivery vehicles, we have tried to obtain them from our Pickering emulsions [14]. For this purpose, the emulsion E MC 2 is dried at 25 °C to remove the continuous aqueous phase and/or the oil-core, and then deposited on an aluminum stub. The SEM pictures, obtained after simple air-drying, show the presence of spherical objects (diameter \approx 7 to 9 μm , see Figure 11A).

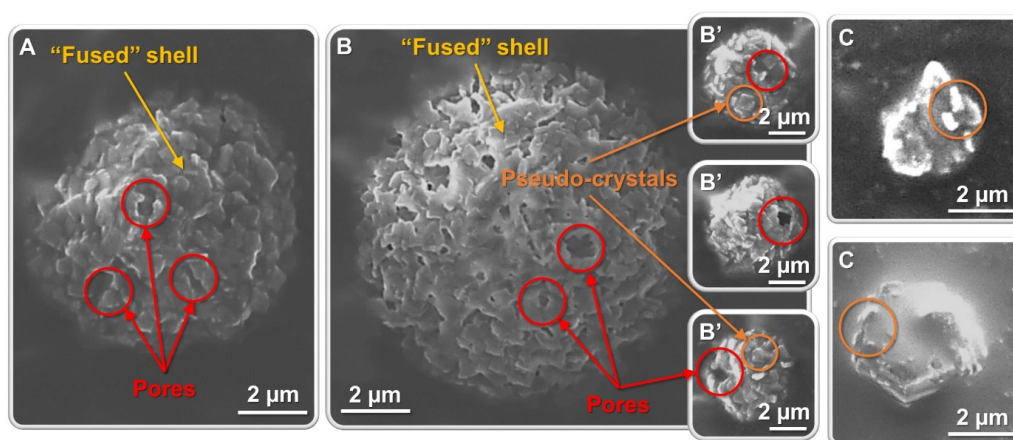


Figure 11. SEM images of microcapsules obtained from emulsions after: (A) evaporation of the water continuous phase by air-drying treatment, (B) evaporation of water by air-drying and partial vacuum treatment to remove a part of oil, (B') total evaporation of water and of oil by air-drying and vacuum treatment, and (C) fast and complete evaporation of the solvents by vacuum treatment.

It is noteworthy that diameters are larger than the paraffin droplets of the emulsion observed with the optical microscope (diameter \approx 3 to 4 μm) due to the aggregation of the PEG/ α -CD nanoparticles. Indeed, a careful look at the droplets surface reveals the presence of "fused" shell made of the PEG/ α -CD nanoparticles (see above). These objects are partially permeable due to the presence of few pores resulting from the fusion of PEG/ α -CD nanoparticles implied in the droplets stabilization (see above). The slow evaporation avoids the crystallization of the nanoparticles and allow the formation of no or weak permeable homogeneous microcapsules in which the MC is solubilized in paraffin oil core. Alternatively, the formation of highly porous capsules can be easily performed by an additional partial vacuum treatment to remove a part of the oil-core at 25 °C (Figure 11B). In these conditions, we obtain slightly expanded capsules around the residual oil-core (diameter \approx 9-12 μm). This expansion is ascribed to the partial evaporation of the oil leading to a clear improvement of the pores number. In contrast, the total evaporation of the oil-core following the air-drying results in compressed microcapsules (Figure 11B'). In such

conditions, the microcapsules size, only loaded with solid MC, is reduced (diameter \approx 4-6 μm) as well as the number of pores. However, this process operates without collapse of structures contrary to direct vacuum treatment. Indeed, we have also performed additional SEM experiments with fast and complete evaporation by vacuum treatment at 25 °C. Due to the brutal vacuum treatment, the droplets are partially destroyed leading to the presence of many collapsed structures. However, some of them retain a more or less spherical shape with diameters smaller than the previous described objects (\approx 2-3 μm , see Figure 11C): these structures are in fact ghost paraffin-droplets. It is noteworthy that the arrangement of these ghost droplets is very similar to the microcapsules but the shell structure is very anarchic as well as the objects shape.

(ii) *Antifungal and antimicrobial activity.* One of the problems associated with the Daktarin® oral gel is that the gel must be kept in the mouth as long as possible before swallowing it. Therefore, solid preparations can be more appropriate to obtain easier-to-follow treatment of fungal infection of the mouth, throat and gut and thus help prevent it coming back. Disk diffusion tests have been performed to evaluate the *in vitro* efficacy of our microcapsules against *C. albicans* and *S. aureus*. For this purpose, as the MCN drug presents the optimal *in vitro* activity against *C. albicans* and *S. aureus* compare to ECN or MC (see Figures 8 and 10), the microcapsules are obtained from the emulsion loaded with 1% of MCN (200 mg) after evaporation of the water continuous phase by air-drying treatment. It is noteworthy that the tablet is moistened with 80 μL of water prior to be placed at the center of the agar plates infected with *C. albicans* or *S. aureus*. The tablet loaded with MCN (prepared from E MCN 1) has been compared to the native emulsion (E MCN 1) used as a reference. The obtained value indicates that, in 48h, the diameters of the zone of inhibition for tablets loaded with MCN (4.2 and 1.8 cm for *C. albicans* and *S. aureus*, respectively) are very similar compared to the emulsion E MCN 1 (3.9 and 1.4 cm for *C. albicans* and *S. aureus*, respectively, see Figures 8 and 10). Similar results are obtained for MC or ECN drugs (MC: 2.5 and 1.4 cm for *C. albicans* and *S. aureus*; ECN: 2.7 and 2.2 cm). Indeed, the release of the azole drugs from the moistened tablets is comparatively similar to that of the native emulsions (compare the previous value with the Figures 8 and 10).

DISCUSSION

This study was undertaken as part of work to develop new formulations that may have application in the management of common skin fungal disorders in the context of greener pharmacy (*i.e.* ethanol, surfactants and silica-free formulations). In order to reduce the human health risks of these excipients, the use of native CDs (α -, β - or γ -CD), derived from biomass feedstocks (starch), is very effective to obtain oil-in-water Pickering emulsions [11,13,14,17-21]. These emulsions, made from biocompatible CDs [22] are stabilized by “particles” made of oil/CD host-guest ICs, following the colloidal tectonics concept, which leads to the hierarchical construction of colloidal systems from molecular building blocks (called tectons) using iterative recognition events between the complementary tectons [8,9]. In our previous work, we have applied these Pickering emulsions as vehicles for ECN

[13]. Despite their high stability, the physicochemical properties as well as the biocidal activity of these emulsions have been shown to be influenced by the CD cavity size. Indeed, the smaller cavities (α - and β -CD) are particularly adapted to the complexation of aliphatic residues unlike the larger γ -CD which is able to accommodate larger substituents such as an optionally substituted aromatic or heterocyclic aromatic group. Consequently, in the presence of γ -CD, the encapsulation of ECN inside γ -CD leads to a slower release of the drug which limits the biocidal activity. Indeed, a partial or total inhibition was observed against *C. albicans* or *S. aureus*, respectively. To minimize the antifungal/CD interactions, the present work was undertaken to obtain topical formulations *via* the use of a pre-assembled system built on α -CD and PEG to obtain acceptable physical and biocidal properties, for further development as a prototype formulation for pre-clinical testing.

We have clearly demonstrated that the α -CD and PEG can easily be used to formulate hydrogels and Pickering emulsions. Indeed, in aqueous solution, α -CD and PEG leads to the formation of hydrogels after heating/cooling cycle. As demonstrated from optical (with or without polarized light) and WETSEM microscopy (Figures 3A, A' and B), the hydrogel structure results of the formation of PEG/ α -CD crystallite made of packed PEG/ α -CD polypseudorotaxane domains in the mixture. These domains act as physical 3D cross-linked networks alongside PEG chains not included within the α -CD cavity. This arrangement is highly compatible with the observations reported in the literature [23-27]. From these hydrogels, oil-in-water Pickering emulsions can then be easily obtained with the mixing of paraffin oil. The optical (with or without polarized light) and WETSEM microphotographs of emulsions (see Figures 3C, C' and D) demonstrate the absence of crystallites in the continuous aqueous phase or onto the droplet surface unlike the optical microphotographs of Potier *et al.* [24]. However, **as the pictures of Potier and coworkers** were observed at 20 °C after a heating at 80 °C, it can be assumed that crystallization is induced by the low evaporation of the organic solvent. Therefore, the crystallites do not seem to contribute to the emulsion stability. Nevertheless, SEM observation of emulsions after drying reveals the presence of numerous submicronic particles (< 500 nm, see Figure 3D'). As a result, we can reasonably suppose that the Pickering stabilization occurs by the way of these nanoparticle-like structures made of aggregated PEG/ α -CD polypseudorotaxanes aggregated. This proposal is in good agreement with the colloidal tectonics concept and the observation made for analogous systems [11-13].

These two systems (hydrogels and emulsions) can be used to obtain antifungal formulations by the dissolution of hydrophobic MC, MCN and ECN drugs. Their weak aqueous solubility is a major problem encountered with hydrogel formulations [2]. As any drug to be absorbed must be present in the form of solution at the site of absorption, the water-solubility enhancement of the azole drugs is required for the direct solubilization in the gels. Among the various methods available, such as the use of surfactants, hydrotropes, co-solvents, we used the highly water-soluble HP- β -CD to increase the drug solubility by complexation [28]. This method avoids the use of a co-

solvent such as ethanol already used in commercial Daktarin® gel. In the literature, some data are available on the inclusion of MCN [2] or MC [16] in HP- β -CD. However, little is known about binding constants for ECN and HP- β -CD. For this reason, the phase solubility diagrams have been investigated. As depicted in Figure 4, the solubility curves are of the type “A_L”, “A_N” and “A_P” for ECN, MCN and MC, respectively. The results are in good agreement with the general observations of Pedersen *et al.* [2]. In all cases, the 1:1 association is prevalent: the binding constants (K₁) increase in the order MC \approx ECN < MCN (Table 1) and the drug solubility is improved in the order MC < MCN < ECN (Figure 4). Noteworthy that the obtained binding constant (K₁) between MCN and HP- β -CD is in good agreement with the published value (280 vs. 306 M⁻¹) [2]. Based on these results, the HP- β -CD was used to dissolve the azole drugs in the hydrogels.

For the various selected formulations presented in Table 2, the physicochemical (texture, pH, microstructures, rheology and stability) and biocidal (antifungal and antimicrobial) properties of loaded hydrogels and emulsions (1 or 2%) have been measured and compared to the commercial references (Micatin®, Pevaryl® and Daktarin®). From the presented results, it is noteworthy that all the formulated hydrogels and emulsions are acceptable for topical applications in term of physicochemical properties. Indeed, the obtained results, for gels and emulsions loaded with drugs, are of the same order of magnitude than the commercial references. However, the ability of the formulated hydrogels to spread is superior (see above). In addition, good long-term stability (> 12 months) is observed for hydrogels: only a small upper water phase is observed corresponding to 0.5% of the initial total volume. For Pickering emulsions, all the physicochemical properties given in Table 3 are close to or even than the data reported in the literature for analogous systems. For instance, Mathapa and Paunov used β -CD to stabilize tetradecane-in-water emulsions (tetradecane oil volume fraction = 0.5) [14]. In such conditions, the average droplet size is estimated around 20 μ m. Moreover, in our previous work, it is noteworthy that the average droplet sizes of paraffin (or isopropyl myristate)-in-water Pickering emulsions stabilized by α -, β - or γ -CD, are comprised between 9 and 16 μ m [13]. Another interesting comparison of our system with that of Marku *et al.* can also be made [29]. In this work, the average mean droplet sizes of Pickering emulsions, containing 56% paraffin oil and 214 mg starch/ml oil, was estimated around 48 μ m. Similar sizes were observed for miglyol and sheanut oil (43 and 73 μ m, respectively). For our Pickering emulsions based on PEG/ α -CD, the quality of the dispersion is clearly better than the reported results available in the literature (between 3.4 and 6.9 μ m) and are of the same order of magnitude than the commercial references (5.3 and 10.1 μ m for Micatin® and Pevaryl®, respectively). Moreover, all the Pickering emulsions stabilized by CDs or starch showed high stability during storage. For instance, Marku *et al.* have reported a very good stability against coalescence over eight weeks and even after mild centrifugation treatment [29]. With regard to the system studied here, very long-term stability (> 12 months) is also achieved without the use of stabilizing or common molecular emulsifying agents. As for other Pickering emulsions, this high stability is one of the major

benefits of these systems. In our case, this property is directly related to the stabilization of the droplets by the aggregated PEG/ α -CD nanoparticle-like arrangements and to the limited-coalescence process. Finally, the Kirby-Bauer disk diffusion susceptibility test against two pathogenic organisms (*C. albicans* and *S. aureus*) show that our formulations are able to inhibit the growth of the studied microorganisms. These *in-vitro* biocidal tests prove that the hydrogels as well as the Pickering emulsions are at least as active as the commercially available formulations. It is noteworthy that, in contrast to our previous study, no partial or total inhibition of the contact biocidal activity against *C. albicans* and *S. aureus*, respectively, is observed with the Pickering emulsions based on PEG/ α -CD due to the absence of azole drug encapsulation inside the α -CD [13]. We even observe that the diameters of the inhibition zone for topical hydrogels are improved compared to the Daktarin[®] reference (up to a factor of 1.6 against *S. aureus*). This observation is very promising for the potential applications of these topical formulations as azole drugs are used to treat jock itch, athlete's foot and oral or vaginal thrush superinfected or not with Gram-positive bacteria.

According to USP regulations [30,31], beside the medical efficiency, the product quality attributes should include but are not limited to the following: description, assay, uniformity and optimal physicochemical properties (*e.g.* apparent viscosity, stability and pH). As previously mentioned, hydrogels and Pickering emulsions based only on two structuring excipients, α -CD and PEG, were found to give optimal homogeneities, viscosities, stabilities, antifungal and antibacterial activities combined with an appropriate pH (see above).

As described in the literature, CD-based Pickering emulsions allow to obtain microcapsules, called cyclodextrinosomes or beads [14,32]. Cyclodextrinosomes are classically prepared from oil-in-water emulsions stabilized by densely packed crystalline structures made of oil/CD ICs. Indeed, CDs complex oil molecules leading to ICs which are self-assembled into colloidal nanoparticles directly onto the oil droplet surface. The desired cyclodextrinosomes are obtained after solvents removal (diameter \approx 6-10 μ m) [14]. From a very close mechanism, beads can be easily obtained thanks to the interactions occurring between CDs and vegetable oils [32]. Indeed, the addition of vegetable oil to a CD aqueous solution results in biphasic system separated by an interfacial film made of triglycerides/CD ICs. After stirring, oil-in-water emulsions are obtained and crystallization of ICs occurs. After few days of continuous stirring, beads are obtained in suspension in an aqueous medium (diameter \approx 0.7-1.6 mm) [33]. Independently to the synthesis methodology, cyclodextrinosomes and beads refer to similar structures resulting from a partially crystalline shell of CD/oil ICs stabilizing oil droplets. The apparent size divergence is only due to the stirring time. Indeed, as beads are obtained after a few days of continuous stirring, the oil droplets become interconnecting *via* the fusion of crystalline areas leading to dispersed droplets in a spherical crystalline matrix of CD/oil ICs [34-36]. These structures are supracolloidal assemblies in the strict sense of that term, since they emerge from the packing of nanoparticles *via* a bottom-up construction. As depicted in **Figure 11A**, the SEM

pictures reveal the possibility to obtain spherical microcapsules (cyclodextrinosomes) loaded with azole drugs (diameter \approx 7 to 9 μm). This size is in good agreement with the literature data [14]. However, the properties of the microcapsules shell can be controlled depending on the drying conditions. Indeed, a fast and complete vacuum treatment results in collapsed microcapsule-like objects (diameter \approx 7 to 9 μm , Figure 11C) whereas air-drying results in a weak permeable shell in which the azole drug is solubilized in paraffin oil core (Figure 11A). Alternatively, the formation of highly permeable capsules can be easily obtained by air-drying and partial vacuum treatment (Figure 11B). In contrast to the literature data, the PEG/ α -CD system allows to control of the properties of the permeability of the microcapsules shell [14,32-36]. Therefore, these microcapsules can be potentially very useful to deliver antifungal medicine. Indeed, some commercial formulations use powders instead of creams or gels. For instance, Daktarin[®] powder 2% is commonly used to treat athlete's foot. This preparation contains MC as antifungal medicine and is very convenient as it allows to sprinkle the powder onto the affected area as well as inside clothing, socks or shoes that are in contact with the infected area to prevent it coming back. The MC loaded microcapsules without paraffin oil could be useful to replace this formulation. Despite the fact that the release of the MCN from the moistened tablets is comparatively similar to that of the native emulsions (see above), this formulation is easier to store, to transport, to administer and to adjust dosages. Finally, it is noteworthy that the microcapsules loaded with MC and paraffin oil can be potentially used to treat fungus infections of covered mucous membranes such as the foreskin of uncircumcised penis. Indeed, after application on the preputial mucosa, the foreskin could be retracting to cover the end of the penis and the MC could be progressively released. Finally, this system could be used to enhance the penetration and absorption of MC with occlusive dressings, which sticks to the skin surrounding the affected area. In this case, the sudation allows to regenerate the emulsion with a progressive MC release.

In the present study, formulations (hydrogels, Pickering emulsions and microcapsules) were prepared for pharmaceutical assessments. If hydrogels and Pickering emulsions present interesting properties in terms of physicochemical and biocidal properties, further studies are now required to measure the stability of the active components in these two formulations, and to examine the influence of factors including temperature, pH and light. For microcapsules, further developments are also required, such as the measurement of the *in vitro* biocidal activity. In addition, for the three formulations, drug release profiles as well as allergy skin tests will be required prior to pre-clinical and clinical trials.

CONCLUSIONS

In this paper, we have formulated several biocompatible drug delivery platforms by using commercially available and non-toxic raw materials (α -CD) and PEG approved by various health authorities (European, American and Japanese Pharmacopoeias). Under appropriate conditions, they are able to produce hydrogels, Pickering

emulsions and microcapsules free of ethanol, surfactants and silica which exhibit some drawbacks towards human health and environment. Getting them is fairly easy, as shown by the encapsulation of antifungal and antibacterial agents, and inexpensive. From the presented results, all the formulated hydrogels and emulsions are acceptable for topical applications (pH, texture and viscosities). The *in-vitro* biocidal tests have demonstrated that the gels as well as the emulsions are able to inhibit fungus and bacterial growth. Moreover, the diameters of the zone of inhibition for topical hydrogels are even improved compared to the Daktarin® commercial reference (up to a factor of 1.6 against *S. aureus*). **In addition, an alternative system based on more or less porous microcapsules derived from emulsions is of potential interest to treat skin and mucous membranes infections with easier to store, to transport, to administer and to adjust dosages than classical oral gel (Daktarin®). Indeed, the microcapsules are able to inhibit fungus and bacterial growth similarly to the native emulsions.** All these systems, stabilized by nanoparticle-like arrangements, made of aggregated PEG/ α -CD complexes, are easily dissociated by topical application or in the presence of physiological fluids without harmful side effects. These systems, obtained according to the so-called colloidal tectonic concept, avoid the use of petro-sourced surfactants or modified silica nanoparticles while keeping at least the same performances and providing solutions to the current trend of simplifying formulas in terms of ingredients. Finally, the presented strategy allows the development of new productive processes by the emergence of a common base of formulation (hydrogels) leading to a set of galenic systems (emulsions and microcapsules). The strategy presented is therefore an invitation to creativity and the ability to innovate in the face of technological complexity and environmental problems.

CONFLICT OF INTEREST

No conflicts of interest to disclose.

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