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Release of antiseptics from the aqueous compartments of a w/o/w multiple emulsion

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Abstract

A w/o/w multiple emulsion drug carrier system has been developed for local vaginal therapy. To improve its efficacy and to extend the antimicrobial spectrum activity of benzalkonium chloride (CBZ), which is introduced in the external aqueous phase, chlorhexidine digluconate (CHD) was added to the internal aqueous phase of the multiple emulsions. The minimal bactericidal concentrations (MBC) for the association of CHD and CBZ in emulsion were determined towards *Escherichia coli* and *Staphylococcus aureus*. The main release mechanism considered for the CHD encapsulated in the inner phase was a swelling-breakdown phenomenon which followed dilution of the emulsion under hypo-osmotic conditions. In order to demonstrate this release, the bactericidal effect of multiple emulsions undiluted and diluted 1–5 and 1–10 in hypo-osmotic conditions at two CHD concentrations was evaluated. To validate and quantify this release, rheological and release kinetics studies were used. The bactericidal activity of combination CBZ–CHD in the emulsion was synergistic on the two bacterial strains and the release of encapsulated CHD in the internal phase was obtained following its dilution in hypo-osmotic conditions. Vaginal administration could be carried out following dilution at 1–5 in sterile water for multiple emulsions containing the lower concentration of CHD.

Keywords: w/o/w emulsions; Synergy; Bactericidal activity; Chlorhexidine digluconate; Benzalkonium chloride

1. Introduction

The antimicrobial properties of a vaginal w/o/w multiple emulsion containing benzalkonium chloride (CBZ) in the outer phase have been demonstrated in a previous study (Tedajo et al., 2002). To improve the performance of CBZ, it may be used in association

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with specific antimicrobial agents such as boric acid, benzylic alcohol and bronopol. These associations allow the extension of the antimicrobial spectrum activity, reinforcement of the antimicrobial properties and a possible reduction in number of doses of active agents (Chevalier et al., 1995; Freney, 1995). In the present study, it was decided to associate chlorhexidine digluconate (CHD) with CBZ. The former is widely used as an antiseptic for vaginal applications, and is characterized by a high level of bactericidal activity and a low level of skin and mucous membrane irritation (Patton et al., 1998). Moreover, CHD is most often found combined with other substances in several pharmaceutical products, notably with quaternary ammoniums (Reverdy, 1995, Reverdy et al., 1996; Pons et al., 1992).

However, CHD can be degraded by light to 4-chloroaniline. This derivative could be toxic towards mucous membranes (Reverdy, 1995, Longworth, 1971). One way to avoid this degradation would be to use multiple emulsions. In fact, many studies in the pharmaceutical, cosmetic and food fields have demonstrated the interest in encapsulating the fragile or biodegradable molecules in the internal phase of these emulsions. Dahms and Tagawa (1996) proposed avoiding further degradation of the labile ascorbic acid against hydrolysis by encapsulating it in polyol-in-oil-in-water multiple emulsions. Tokgoz (1996) had also studied the encapsulation of ascorbic acid in the inner phase of a multiple emulsion. Cunha et al. (1997) have shown in vitro that multiple emulsions were able to protect insulin against enzymatic degradation. Therefore, in this study CHD was encapsulated in the inner phase of w/o/w multiple emulsions to protect against light degradation. The release of the CBZ takes place immediately after vaginal administration of multiple emulsions. In fact, its localisation in the outer phase causes it to be so available. On the other hand, the release of CHD encapsulated in the inner aqueous phase is only possible after dilution of the preparation. Therefore, in order to be available as CBZ at the moment of vaginal use, dilution of the emulsion in pure water was envisaged. The concentration gradient due to the dilution induces a water flow from the external to the internal phases and then the swelling of oil globules followed by their breakdown and the release of entrapped substances (Florence and Whitehill, 1981). Without considering the protection of CHD against light degradation (which could be realized by other solutions), CHD could be considered as a model of molecule which has to remain encapsulated during storage and to release at the very moment of the vaginal application after dilution, according an original controlled release mechanism.

The objective of the present work was:

- Firstly, to determine the minimal bactericidal concentration (MBC) of individually or mixed antimicrobial substances in solution and in multiple emulsions, to evaluate antimicrobial interaction of the combination of the two compounds;
- Secondly, to demonstrate the release of CHD by the swelling-breakdown mechanism, through the evaluation of the bactericidal effect of undiluted and diluted multiple emulsions; and
- Thirdly, to carry out a well-controlled physicochemical method to verify that the release mechanism of CHD is actually induced by swelling-breakdown.

2. Materials and methods

2.1. Preparation of multiple emulsion

The following substances were used in the preparation of the emulsions: the aqueous phase was deionized water. The oil used was Parleam® (hydrogenated polyisobutene, Rossow et Cie, Levallois-Perret, France). Abil EM 90® (cetyl dimethicone copolyol, Goldschmidt, Montigny-le-Bretonneux, France) was used as the lipophilic surfactant, Synperonic PE/F127® (ethylene and propylene oxide copolymer, Uniquema, Paris, France) was used as the hydrophilic surfactant. Sodium chloride (Prolabo, Nogent, France) was used as the swelling-breakdown marker. Benzyldimethyl tetradecylammonium chloride (CBZ) (Fluka, Saint-Quentin Fallavier, France) and aqueous solution of CHD at 20% (w/v) (Fluka, Saint-Quentin Fallavier, France) were used as the active substances. w/o/w multiple emulsions were prepared using a two-step process (Raynal et al., 1993). The composition of the different emulsions is presented in Table 1.

Table 1
Composition of multiple emulsions (%, w/w)

	A	B	C	C′	D	D′
Composition						
Primary w/o emulsion						
Polyisobutene	24	24	24	24	24	24
Cetyldimethicone copolyol	6	6	6	6	6	6
Chlorhexidine digluconate (20%)	–	–	0.625	1.25	0.625	1.25
Chloride sodium	0.5	0.5	0.5	0.5	0.5	0.5
Desionised water(fill up to)	100	100	100	100	100	100
Multiple w/o/w emulsion						
Primary emulsion	80	80	80	80	80	80
Benzalkonium chloride	–	0.2	–	–	0.2	0.2
Ethylene and propylene oxide copolymer	0.8	0.8	0.8	0.8	0.8	0.8
Desionised water(fill up to)	100	100	100	100	100	100
Characterisations						
Multiple globule size (μm)	5–10	3–5	8–10	10–15	5–10	5–10
Conductivity (μS/cm)	74.2	71.3	37.3	39.0	55.1	64.9
Yield (%)	90.5	91.5	99.4	99.0	95.3	92.9
Centrifugation stability	Stable	Stable	Stable	Stable	Stable	Stable

2.2. Characterization of multiple emulsions

Macroscopic analysis was carried out in order to observe the homogeneity of the different emulsions.

Microscopic analysis was performed using an optical immersion microscope (Olympus BX 60, Rungis, France) connected to a video camera at 100× magnifying power, in order to measure globule size.

Assessment of multiple emulsion stability (prevention of creaming, absence of breakdown) was evaluated by the centrifugation test. This assessment was performed immediately after preparation using a centrifuge (Jouan, Paris France) for 15 min at a centrifugal acceleration of $2400 \times g$ at $20 \pm 1\,^{\circ}C$.

Assessment of the encapsulation rate of the w/o/w multiple emulsions was determined by conductimetric tests, and carried out using a CDM 230 conductimeter (Tassel, Radiometer, Copenhagen, Villeurbanne, France) at $20 \pm 1\,^{\circ}C$ on 1/20 diluted emulsions with an aqueous solution of glucose (4%, w/v). The glucose solution has the same osmolarity as the inner phase (232 mOsm) and allows the structure of multiple emulsions to be preserved. This test permits the measurement of the weight fraction $\beta(t)$ of the electrolyte NaCl released into the external aqueous phase at a given time t:

$$\beta(t) = \frac{M(t)}{M_0}$$

where M_0 is the initial amount incorporated and $M(t)$ is the amount present in the external phase at a given time t. The $\beta(t)$ value gives the entrapment rate Y of the multiple emulsions according to the relationship:

$$Y = 1 - \beta(t)$$

The released mass fraction of electrolyte was obtained from a calibration curve of a diluted multiple emulsion.

The characteristics of the emulsions were determined immediately after preparation and after nine months on samples kept at $20 \pm 1\,^{\circ}C$.

2.3. Microbiological evaluation

2.3.1. Assessment of minimal bactericidal concentration

Assessment of minimal bactericidal concentration of the active substances in solution and in multiple emulsions was based on the European Standard NF EN 1040 (Anonymous, 1997). These tests were carried out in suspension using the dilution neutralization method. Multiple emulsions were diluted in sterile distilled

water (a hypo-osmotic condition) in order to allow the release of CHD from the internal phase. Diluted emulsions were maintained at room temperature for 6 h (De Luca, 1991). For the contact emulsion-bacteria, as in the previous study (Tedajo et al., 2002), gentle agitation was achieved by simply inverting the tube. The bacterial strains selected for the tests were *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 10536. The neutralizing diluent used after validation was a sterile solution of polysorbate 80 (6%, w/v), lecithin (0.6%, w/v), L-histidine (0.1%, w/v), chloride sodium (0.43%, w/v) and casein peptone (0.1%, w/v), in a pH 7.0 phosphate buffer (3.56 g KH_2PO_4, 7.23 g $Na_2HPO_4{\cdot}2H_2O$ per litre distilled water).

For each test, the MBC was determined. The MBC is defined as the lowest concentration at which the product tested is capable of reducing by at least 10^5, the number of viable bacterial cells within 5 min of microbial-product contact.

The reduction was evaluated by the following way: N_0/N_x, where N_0 was the number of viable cells per millilitre in the test mixture at the beginning of the contact time "zero", and N_x was the number of survivors per ml in the test mixture at the end of the contact time and before neutralization. The MBCs were expressed in % (w/v) of the emulsion studied.

To determine the type of interaction that occurs between CHD and CBZ in aqueous solution, different concentrations of CHD (MBC, MBC/2, MBC/4, MBC/8) were added for each fixed concentration of CBZ. The bactericidal activity of these binary combinations was then evaluated in suspension using the dilution neutralization method as described above. Following the method used by Berenbaum (1978), each result was expressed as the fractional bactericidal concentration index (ΣFBC), which is the sum of the lowest concentration of each compound of the solution or emulsion which reduced, by at least 10^5, the number of living bacterial cells (expressed as a fraction of MBC). The ΣFBC values allowed interpretation according to the following criteria: $\Sigma FBC \leq 0.75$: synergistic; $\Sigma FBC = 1$: additive; $1 < \Sigma FBC < 2$: indifferent; $\Sigma FBC \geq 2$: antagonistic.

2.3.2. Microbiological study of CHD release

In order to provide a driving force for CHD release, multiple emulsions which only contains CHD were diluted under hypo-osmotic conditions using sterile water, either at 1–5 or 1–10, and maintained at room temperature for 6 h. After this time, the undiluted and diluted multiple emulsions were inoculated with the cell suspension ($1–3 \times 10^8$ CFU/ml). Inoculation was carried out at a level of 50 μl of cell suspension per 5 g of emulsion. The inoculated emulsions contained between 1×10^6 and 3×10^6 CFU/g. Emulsions were sampled after 5 min of contact then inactived and diluted before being spread on agar plates in order to obtain a bacterial count. Inactivation was carried out using a neutralizing diluent. The bacterial strains and neutralizing agent used are described above in Section 2.3.1.

The reduction of the viable population was determined by the following equation:

$$R = \log T_0 - \log T_{5\ \mathrm{min}}$$

$\log T_0$ and $\log T_{5\,\mathrm{min}}$ were, respectively, the log of the number of viable microorganisms before starting the test (determined by the viable population of inoculum) and that after 5 min of product–bacteria contact.

2.4. Physico-chemical study of CHD release

2.4.1. Swelling-breakdown mechanism

The swelling and breakdown was evaluated by rheological analysis. This test was performed at 20 ± 1 °C using a controlled-stress rheometer, Carri-Med CSL 100 (Rheo Palaiseau, France), with a cone plate geometry (d = 60 mm; $\theta = 2.02°$; gap = 60 μm). In this experiment, the diluted sample (1–5) in hypo (desionised water) and iso-osmotic conditions (aqueous solution of glucose) was subjected to a constant shear rate ($100\ s^{-1}$) and the evolution of the viscosity η was recorded (Fig. 1). This analysis gave information on the evolution of the volume fraction of the multiple emulsions during the time period (Grossiord et al., 1993; Terrisse et al., 1994).

2.4.2. Release kinetics studies

NaCl was used as a marker to quantify the breakdown. The release rate of this marker was evaluated by conductivity measurement after dilution of the sample (1–5, 1–10 and 1–20) in iso-osmotic and in hypo-osmotic conditions.

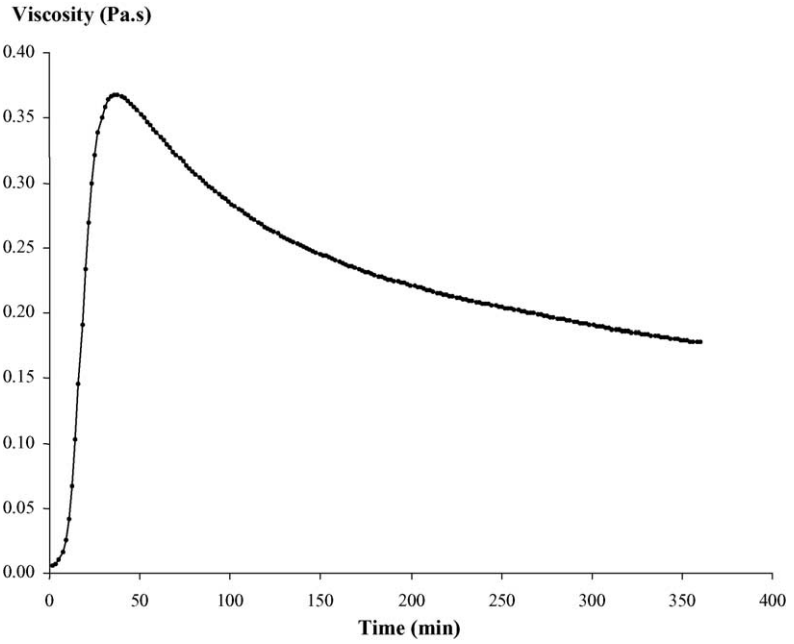

Fig. 1. Graphs of viscosity versus time for hypo-osmotic dilutions (five-fold) for multiple emulsion C.

3. Results

3.1. Characteristics of multiple emulsions

All emulsions were macroscopically homogeneous. The conductivity of multiple emulsions (measured after preparation) corresponded to a high encapsulation rate of NaCl (over 90%) for all emulsions (Table 1). The encapsulation rate and the globule size were particularly important for emulsions C and C′. Emulsions D and D′ stayed stable and homogeneous after nine months of storage. Moreover, the conductimetric measurement did not show any significant change in the fraction of NaCl release from the emulsions D and D′ (values of rate encapsulation were, respectively, equal to 95.3 and 92.2 μS).

3.2. Microbiological evaluations

3.2.1. In vitro assessment of minimal bactericidal concentration

The MBCs of CBZ obtained in solution were, respectively, 0.002–0.004% (w/v) and 0.001–0.004% (w/v) for *E. coli* and *S. aureus*. For CHD, the MBCs were, respectively, 0.004–0.008% (w/v) and 0.016% (w/v) for *E. coli* and *S. aureus*. The combination of the two antimicrobial agents (CBZ and CHD) in solution was synergistic towards *E. coli* and *S. aureus* ($\Sigma FBC \leq 0.75$) (Tables 2a and 2b).

Bactericidal activity of the control emulsion A formulated without CBZ and CHD was inactive towards both strains (data not shown). The MBC of emulsion multiple B containing CBZ in the outer phase was respectively two- to four-fold lower for *E. coli* than those obtained for CBZ solution (Table 3). However, the MBCs of CBZ in solution and in emulsion were similar for *S. aureus*. Multiple emulsion C containing CHD at 0.1% in the inner phase was inactive towards *S. aureus* for the greater concentration of emulsion tested (20%), but active towards *E. coli*. A doubled concentration of CHD (emulsion C′) induced a bactericidal activity on the two strains. Multiple emulsions D and D′ containing CBZ in the outer phase and CHD in the inner phase at respective concentrations of 0.1 and 0.2% had a high level of bactericidal activity on *E. coli* and *S. aureus*. The combined effect produced by CBZ–CHD in emulsions (D and D′) was greater than the sum of the individual effects of either active substance alone

Table 2a
Reduction of viable microorganisms of the combination CHD + CBZ in solution against *E. coli* ATCC 10536

Chlorhexidine digluconate (CHD)	Benzalkonium chloride (CBZ)				
	0	MBC, 0.004%	MBC/4, 0.001%	MBC/8, 0.0005%	MBC/16, 0.00025%
0		>6.0			
MBC, 0.008%	>6.0	–	–	–	–
MBC/2, 0.004%			>6.0, FBC = 0.75	3.57	3.04
MBC/4, 0.002%			3.26	<2.55	<2.55
MBC/8, 0.001%			3.01	<2.55	<2.55

Values are means ($n = 2$).

Table 2b
Reduction of viable microorganisms of the combination CHD + CBZ in solution against *S. aureus* ATCC 6538

	Benzalkonium chloride (CBZ)			
	0	MBC, 0.001	MBC/2, 0.0005	MBC/4, 0.00025
Chlorhexidine digluconate (CHD)				
0		>6.10		
MBC, 0.016	–	–	–	–
MBC/2, 0.008		>6.10	>6.10	3.99
MBC/4, 0.004		>6.10	5.79, FBC = 0.75	4.97
MBC/8, 0.002		>6.10	>6.10, FBC = 0.625	3.95

Values are means ($n = 2$).

in emulsions (B, C and C′). Indeed this combination was synergistic ($\Sigma FBC \leq 0.75$).

3.2.2. Microbiological study of CHD release

For the undiluted emulsion C, the reduction of viable microorganisms was very low whatever the bacterial strain tested (Table 4). A greater reduction was observed for the diluted multiple emulsion C (1–5, 1–10) on *E. coli* but not enough to obtain the MBC. A less significant reduction of *S. aureus* was obtained with the diluted emulsions, compared to that obtained for *E. coli*. The reduction stayed low for the undiluted emulsions C′. However, a significant reduction in both strains was obtained for the diluted emulsion C′, and

Table 3
Determination of minimal bactericidal concentration of CBZ and/or CHD and fractional bactericidal concentration of the combinations CHD + CBZ in emulsion

	B	C	C′	D	D′
E. coli ATCC 10536					
MBC of multiple emulsions (%, w/v)	4	20	2.5	0.625	1.25
Active substances[a] (%, w/v)					
CBZ	0.008			0.000625	0.00125
CHD		0.02	0.005	0.00125	0.0025
S. aureus ATCC 6538					
MBC of multiple emulsions (%, w/v)	1	Inactive[b]	20	0.3125	0.625
Active substances[a] (%, w/v)					
CBZ	0.002			0.0003125	0.00125
CHD			0.02	0.000625	0.000625

Values are means ($n = 3$). B: 0.2% CBZ; C: 0.1% CHD; C: 0.2% CHD; D: 0.2% CBZ + 0.1% CHD; D: 0.2% CBZ + 0.2% CHD.
[a] MBC of active substances in considered emulsion.
[b] Inactive for the concentration tested (20, 10, 5, 2.5, 1.25).

Table 4
Evaluation of the bactericidal effect for undiluted emulsion, emulsion diluted at 1–5 and emulsion diluted at 1–10, towards two strains

Emulsions	T_0 log (CFU/g)	Reduction of viable microorganisms		
		Emulsion undiluted	Emulsion diluted at 1/5	Emulsion diluted at 1/10
E. coli ATCC 10536				
C	6.1 ± 0.1	1.1 ± 0.4	4.2 ± 1.1	4.1 ± 0.7
C′	6.2 ± 0.2	1.9 ± 0.6	≥ 5.0	≥ 5.0
S. aureus ATCC 6538				
C	6.3 ± 0.1	0.49 ± 0.3	2.2 ± 0.5	3.4 ± 0.4
C′	6.3 ± 0.06	1.6 ± 0.9	4.2 ± 0.6	3.4 ± 0.4

Values are means ($n = 3$).

one slightly higher than that obtained for emulsion C. No difference in this reduction of *E. coli* was observed between the two dilution ratios. A slight difference was observed for *S. aureus*.

3.3. Physico-chemical study of CHD release

3.3.1. Swelling-breakdown mechanism

This study was carried out in order to demonstrate the release mechanism of CHD from the inner phase of a multiple emulsion.

(1) In the case of iso-osmotic dilution, the viscosity remained almost constant over time (about 0.003 Pa s) showing an absence of swelling multiple droplets.
(2) In the case of hypo-osmotic dilution in purified water the concentration gradient between all the dissolved species $C > 0$ was responsible for water flow from the external phase to the internal phase. This aqueous transport produced an increase in internal microglobule size and consequently the oil globules swelled until a critical size was obtained. This swelling step caused an increase in viscosity to a maximal value (0.37 Pa s). Beyond this critical size, the globules burst as a result of the breakdown of the oily membrane and the release of entrapped substances. This breakdown step lead to a decrease in viscosity (0.18 Pa s after 6 h). Similar kinetics of swelling-breakdown were obtained with emulsion C′ (data not shown).

3.3.2. Release kinetics study

This study was carried out in order to quantify breakdown release. The extremely hydrophilic nature of the electrolyte (NaCl) prevents it from crossing through an oily membrane by simple diffusion. But some authors (Omotosho et al., 1986) reported that it can cross by diffusion in inverse micelles in the oily phase. However, the polymeric surfactants which were used (Abil 90 and Synperonic PE/F127) do not seem to be able building such inverse micelles, due to their high molar mass, which probably makes impossible this micellar diffusion (Sela et al., 1993; Jager-Lezer et al., 1997). NaCl was thus considered as a breakdown indicator, which indirectly reflected the release of CHD from the internal phase. The release profiles of NaCl from emulsions C and C′ diluted in hypo-osmotic conditions were similar and only the results for C are shown in Fig. 2: in iso-osmotic conditions there was no significant release (data not shown). On the other hand, in hypo-osmotic conditions NaCl encapsulated was released from emulsion C after 3 h. This release was dependant on the dilution ratio: the release of NaCl encapsulated from the inner phase of the multiple emulsion was 40, 60 and 70, respectively, for 1–5 dilution, 1–10 dilution and 1–20 dilution. A greater release for the higher dilution ratio has also been found for the multiple emulsion C′.

The results showed a time release which was typically of about 100 min (Fig. 2). So it seems logical to consider that a significant mucosal absorption of water could be happen during this time. Moreover, the release resulted in reduction of the concentration gradient between the aqueous phases. In both cases, the osmotic aqueous flow will be reduced and therefore the rate of release. In order to define a controlled release system adapted to these changing conditions, it will be necessary to take particular care on the formulation and dilution parameters, according the application.

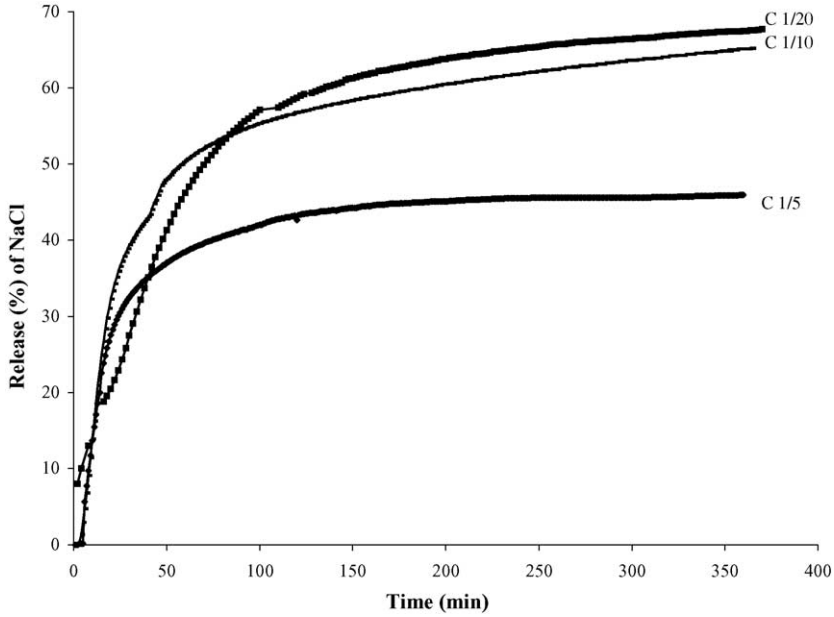

Fig. 2. Release of NaCl from multiple emulsion C diluted in hypo-osmotic conditions.

4. Discussion

Benzyldimethyl tetradecylammonium chloride (CBZ) was introduced in the outer phase of a w/o/w multiple emulsion and associated with chlorehexidine digluconate (CHD). This last molecule was encapsulated in the inner aqueous phase of the w/o/w multiple emulsion to protect it against an eventual degradation. The in vitro MBC of the two antimicrobial agents, individually and in association, in solution and in emulsion, was determined. This study showed that a combination of CBZ–CHD in solution was synergistic towards *S. aureus* and *E. coli*. Similar results have been also obtained by other reports (Pons et al., 1992; Chevalier et al., 1995). It was noted that the activity of CBZ in solution was greater than the activity of CBZ in emulsions for *E. coli*, but not for *S. aureus*. It is probable that one of the excipients adheres to the surface of the Gram-negative bacteria and reduces the fixation of CBZ. In the same way, CHD in solution showed a greater activity on the two strains than CHD in emulsion. In this case, the lower activity in emulsion could be attributed to an interference between CHD and one of the excipients or to the amount released from the multiple emulsions: only 40 and 60% of the active substances were released from the multiple emulsion C at, respectively, 1–5, 1–10 dilutions. Despite the lower activity of multiple emulsion containing CHD, the combination of active substances (CBZ and CHD) in emulsion D and D′ for the two tested strains have presented a synergistic activity on *S. aureus* and *E. coli*. Such results were also obtained by Chevalier et al. (1995) who demonstrated the synergistic activity of the combination of CHD and CBZ in an antiseptic formulation towards *E. coli* and *S. aureus*. The results of the first part of the present study justify the incorporation of these two active substances in the same system. Increasing the concentration of CHD in the combination CBZ–CHD (emulsion D′) does not enhance the bactericidal activity of emulsions. Consequently, the formulation D will be retained for an in vivo study.

The CHD encapsulated in the inner phase must be available, just as the CBZ is available, at the moment of vaginal application. Consequently, the CHD must be released from the inner aqueous phase of an emulsion prior to administration. In the literature on the subject, two main release mechanisms are widely cited as possible methods of delivery for a water-soluble drug from the inner phase of multiple emulsions (Jager-Lezer et

al., 1997; Grossiord and Seiller, 2001). Release could be induced either by transport through the membrane, or by membrane breakdown (following swelling or shearing). It can be strongly supposed that CHD is hydrophilic enough to prevent its crossing the oily membrane by simple diffusion. Moreover, as noticed previously, it is likely that the diffusion by micellar route can be excluded, due to the polymeric nature of surfactants. Therefore, release of CHD by the first mechanism was unlikely. In any case, even if CHD would have some affinity for the oil, its transport by Fick's diffusion into the external aqueous phase would be relatively slight, due to the high volume fraction of the multiple emulsion before dilution.

Consequently, the breakdown of the oily membrane following swelling was considered for the release of CHD. This mechanism was more suitable for the vaginal route compared to the breakdown that occurs under shearing. Taking into account the high osmolarity and the low volume of vaginal secretions, about 0.5–1 ml (Guyot and Fawaz, 1993), the multiple emulsions must be diluted in pure water before administration. The bactericidal effect has been assessed for 1–5, 1–10 diluted emulsion in hypo-osmotic conditions and compared to that of undiluted emulsions in order to demonstrate the swelling-breakdown mechanism. Our results seem to show that this mechanism was indeed involved. A very low reduction of viable bacterial cells was obtained for undiluted multiple emulsions which contained only CHD as an active substance (C and C′). However, a considerable reduction was found for the diluted emulsions, confirming the release of CHD from the inner phase of multiple emulsions. The low activity of undiluted emulsions could be attributed to the oily droplet volume fraction, which is near to 80%. At such high concentrations the configuration of the dispersed drops is closely packed and consequently provides a great stability (Terrisse et al., 1994). Therefore, the undiluted emulsion structure prevents the release of the active substances for as long as they are stored. The reduction of viable microorganisms for the diluted emulsions C and C′ was not significantly different between the two dilution ratios (1–5, 1–10). This fact could be explained by release kinetics, which demonstrate the greater release of the marker at higher dilution, compensating for active substance dilution. The rheological analysis and release kinetic studies of multiple emulsion C allowed us to validate and to quantify the release by swelling-breakdown. The change in viscosity versus time obtained in hypo-osmotic conditions was characteristic of the swelling-breakdown mechanism. The release of NaCl, which indirectly reflects the release of CHD from the inner phase of multiple emulsions, was exclusively obtained in hypo-osmotic conditions. For the in vivo study, the chosen formulation could be diluted five-fold in sterile water prior to vaginal administration.

5. Conclusion

For vaginal delivery, stable w/o/w multiple emulsions containing chlorhexidine digluconate in the internal phase and benzalkonium chloride in the external phase were obtained. Assessment of the minimal bactericidal concentration for chlorhexidine digluconate and benzalkonium chloride in emulsions under hypo-osmotic conditions demonstrate the synergistic activity of the combination. Evaluation of the bactericidal effect has shown that a swelling-breakdown process could induce release of chlorhexidine digluconate encapsulated in the internal phase, following dilution of multiple emulsions under hypo-osmotic conditions. The results obtained from release kinetics studies confirmed the involvement of this mechanism. Consequently, the dilution of emulsions in water is essential prior to vaginal administration. Therefore, this study demonstrated the applicability of w/o/w multiple emulsions as a new drug carrier system for vaginal delivery.

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