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Adsorbed pluronics on the skin of human volunteers: effects on bacterial adhesion

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Abstract

An amphiphilic copolymer, Pluronic F127, has been deposited, by adsorption, to the skin of human volunteers and the ability of the coated skin to resist bacterial colonisation has been evaluated. In parallel, the ability of the same copolymer to act as a bacterial release agent has been evaluated. In both cases, F127 proved to be of little added value in formulations designed to suppress the bacterial colonisation of human skin.

Keywords: Bacterial-release coatings; Pluronic F127; In vivo evaluation

1. Introduction

As an integral part of their survival mechanism, bacteria have a natural tendency to adhere to surfaces (Costerton and Lappin-Scott, 1989). In many environments, long-term adhesion and the development of bacterial colonies or biofilms can result in contamination, infection and disease. As a result, the inhibition of bacterial adhesion to surfaces is of significance to many industrial technologies, medical care and food processing (Zottola and Sasahara, 1994; Mafu et al., 1991; Bower et al., 1996; Cunliffe et al., 1999). Consequently, there has been a long-standing interest in devising methods for the control, prevention or retardation of biofilm formation: free or immobilised antibiotics (Josefsson et al., 1981; Greco et al., 1982); polymer-bound biocides (Deitch et al., 1983); covalently bonded antiseptic agents (Ikeda et al., 1986); and quaternary amine-containing organosilicone salts (Speir and Malek, 1982); have all been examined as possible means of rendering bacterial colonies inactive.

As an alternative to the biocidal approach, surface modification provides a non-toxic means to the inhibition of the bacterial colonisation of

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surfaces. In this respect, two main strategies have emerged as potential means of producing bacterial-resistant surfaces, namely: (i) the hydrophilic approach; and (ii) more recently, the low surface energy approach (Tsibouklis et al., 1999).

Adsorbed, surface-displayed amphiphilic block or graft co-polymers capable of forming pendant chain barriers are known to minimise protein and cellular adhesion (de Gennes, 1987; Taunton et al., 1988; Jeon and Andrade, 1991; Jeon et al., 1991; Tadros and Vincent, 1980). In a systematic study (Bridgett et al., 1992), a range of commercially available Pluronic surfactants were adsorbed onto model polystyrene surfaces, and the potential of the modified surfaces to control the adhesion of three well-characterised clinical isolates of *S. epidermidis* was examined. These workers also considered the significance of the chain lengths of the constituent poly(propylene), PPO and poly(ethylene oxide), PEO, constitutional repeat units in determining adhesion control. Reduction in bacterial adsorption onto polystyrene model surfaces was found to be effected by Pluronic molecules having PPO chains varying between 16 (P35 and F38) and 67 (F127) constitutional repeat units. The results further revealed that for a fixed length of the anchoring PPO chain (Pluronics from L61 to F68), varying the PEO chain lengths from 3 to 76 monomer units did not alter the anti-adhesion capability of the co-polymer system.

The aim of this study is to evaluate—through the use of human volunteers—the in vivo performance of one of the polymers identified by Bridgett et al. (1992) as efficient bacterial adhesion mediators, namely, Pluronic F127.

2. Experimental

2.1. Materials and methods

2.1.1. Chemicals

Poly(ethylene oxide)-co-poly(propylene oxide) (PEO–PPO) co-polymers (Pluronics^{TM}) were obtained from BASF. For the in vivo experiments 'Dettol® Antimicrobial/bacterial Liquid Hand Wash' ('Dettol wash'; Reckitt Benckiser) was used: this contains tartrazine, sodium chloride, and the antibacterial ingredient *para*-chloro-*meta*-xylenol, PCMX; dettol/pluronic formulations were prepared by simple mixing of the commercially available Dettol® product with an aqueous solution of F127 (2% w/v; sterilised by autoclaving at 121 °C for 20 min) in the proportions summarised in Table 1. Maximum recovery diluent (MRD, Oxoid, containing peptone (1 g l^{-1}) and sodium chloride solution (8.5 g l^{-1}) in aqueous buffer pH 7.0 $\pm$ 0.2) and Triton X-100 (t-Octylphenoxypolyethoxyethanol, non-ionic surfactant) were used for serial dilutions.

2.1.2. Growth of organisms

Serratia marcescens is an enteric gram-negative rod not usually found on human skin but which-due to its pink colouration on incubation at ~ 35 °C—provides a readily accessible marker organism against the background of skin commensals, was employed in this study. Cultures of *S. marcescens* (typically 10^8 organisms ml^{-1}) were grown statically in nutrient broth (Oxoid) for 24 h at 37 °C.

2.1.3. In vivo assessment

Ethical approval was obtained to conduct experiments to investigate treatment of human skin with wash products containing copolymer F127 and to evaluate the effect of these treatments on bacterial adhesion over specified time periods. All human volunteers were screened so as to exclude from the study any with cuts or grazes on or around the area of treatment. Participants were requested not to use skin creams or cosmetics for 24 h prior to each experiment.

2.1.4. Part 1: treatment of human skin with (i) F127 copolymer (ii) dettol wash, and (iii) a dettol formulation incorporating F127; effects on skin commensals

The study was carried out on the four fingers (not thumbs) of both hands of six volunteers.

Both hands of each volunteer were rinsed under running deionised water (~ 1000 ml) and allowed to dry in air (no forced convection). The fingers were dipped (1 min) into the appropriate solution, Table 1(a), contained in a pre-sterilised bottle and allowed to dry. The underside tip of each finger

Table 1
(a) Treatments; (b) variables (Factors) included in assay; (c) pre-treatments; (d) variables (Factors) included in pre-treatment assay; and (e) post-treatment

(*a*)

Treatment number corresponding to fingers	Pre-treatment applied to fingers (sterilised; 121 °C, 20 min)
1 index	Deionised water
2 middle	2% w/v F127
3 ring	2% w/v F127 in 2% w/v dettol wash
4 little	2% w/v dettol wash

(*b*)

Variable (factor)	Level
Dettol wash	With/without
Copolymer F127	With/without
Person	Six individuals
Hand	Two hands
Digits	Eight fingers

(*c*)

Pre-treatment number	Pre-treatment applied to the skin of the arms
1	Deionised water
2	2% w/v copolymer F127
3	2% w/v copolymer F127 in 50% w/v dettol wash
4	50% w/v dettol wash

(*d*)

Pre-treatment variable (Factor)	Level
Dettol wash (50% w/v)	With/without
F127 copolymer (2% w/v)	With/without

(*e*)

Treatment number	Treatment applied to skin
1	Deionised water
2	2% w/v F127
3	2% w/v F127 in 50% w/v dettol wash
4	50% w/v dettol wash

was gently rolled onto a nutrient agar plate, which was incubated overnight (37 °C) to encourage growth of any residual skin flora deposited onto the plates. Each experiment was repeated four times for every volunteer, yielding 32 observations. The treatments to the fingers were rotated with each experiment so that the eight fingers of each volunteer formed an experimental 'block'—i.e. provided a group of data containing all the variables in terms of skin treatment. The number of colony-forming units (CFU) on each agar plate was counted using the optical microscope. For the analysis of variance, each variable in the experiment was assigned as a 'Factor', Table 1(b). The data (CFU per plate) were analysed using SUPER ANOVA software (Abacus Systems, 1998).

2.1.5. Part 2: S. marcescens adsorption assay on human skin pre-treated using (i) F127 copolymer (ii) dettol wash, and (iii) a dettol formulation incorporating F127

This study was performed according to the method of Williamson and Kligman (1965); three human volunteers were employed. Both arms of each volunteer were rinsed in running de-ionised water (~ 1000 ml) and allowed to dry. Four circles (area 4.91 cm^2) were marked out on the underside of the skin of each arm. Sterile glass rings (area 4.91 cm^2) were held in position, on the arm, to contain liquid samples and suspensions that were subsequently applied to the skin. The pre-treatment solutions (1 ml, Table 1(c)) were applied to the skin for 1 min, after which time the skin was rinsed under running water (~ 1000 ml) and allowed to dry.

The culture of *S. marcescens* (1 ml) was placed on a predefined area of skin (4.91 cm^2) where it was allowed to stand for 5 min before being removed with a pipette. The skin was rinsed with sterile, de-ionised water (~ 500 ml, 1 min) and the rinsings were collected. A solution of phosphate-buffered saline (PBS) and Triton X-100 (1 ml) was then applied to the skin and the area agitated with a smooth-ended glass rod (1 min). The liquid was removed using a pipette and placed in a dilution tube. The last step was repeated, and the two samples were combined. Serial dilutions of the collected rinsings and also of the washings taken from the skin (in PBS and Triton X-100) were carried out in MRD. Samples from each dilution (100 μl) were plated out and incubated overnight (35 °C) to facilitate enumeration.

As with the previous set of experiments, the four areas marked out on each of the two arms formed an experimental 'block'—i.e. a group containing all of the treatments; the treatment regime imposed on each area was rotated with each experiment. The number of organisms per treatment area was determined by counting the number of CFU on each agar plate. In order to allow for the large number of variables inherent to this assay—for example, subject to subject variations, imprecise volume and flow control—the data were considered in the light of multi-variate analysis, Table 1(e).

2.1.6. Part 3: adsorption assay on human skin exposed to cultures of S. marcescens and subjected to post-exposure treatment with (i) F127 copolymer (ii) dettol wash, and (iii) a dettol formulation incorporating F127

Four human volunteers were recruited for this study. Each arm was rinsed with deionised water ($\sim$1000 ml) and allowed to dry. Treatments, contained in sterile vessels, were then applied to the skin (4.91 cm^2) according to the method described for the previous set of experiments.

A culture of *S. marcescens* was allowed to colonise the skin of human volunteers. To facilitate colonisation, a predetermined volume (1 ml) of the bacterial culture in nutrient broth (10^8 organisms per ml) was allowed in contact with skin for 5 min. After this time, the skin was rinsed with water (1 min) and the post-treatment under consideration (1 ml; Table 1(f)) was applied (1 min) by inverting the vessel onto the marked area of skin, care been taken to avoid the spillage of fluid. Following exposure to the post-treatment, the sample bottles were returned to the upright position—by rotating the arm while holding the vessels in place—and, by means of serial dilution, the contents were assessed for the number of organisms not bound to the skin i.e. those remaining in suspension. A solution of PBS+ Triton X-100 was then applied to the skin (1 ml), the area was agitated with a smooth-ended glass rod (1 min) and the fluid was removed with a pipette before being placed in a dilution tube; the last step was repeated and the second sample was combined with the first. Serial dilutions of the organisms collected from the skin, and also those of organisms in the post-treatment sample bottles, were carried out in MRD. Aliquots of resulting suspensions (100 μl) were applied to agar plates, which were incubated overnight (35 °C) and the number of CFU was recorded for each plate. The numbers of CFU recorded per plate were related to each skin post-treatment regime and assay variable using ANOVA multi-variant data analysis. As with the previous sets of experiments, each variable was assigned as a Factor, and its overall effect on the number of adsorbed organisms was quantified.

3. Results and discussion

3.1. The effects of dettol products, and F127 copolymer, on microbial adhesion to human skin

Polymeric surfaces, which have been modified through exposure to solutions of the PEO–PPO copolymer 'Pluronic F127', are known to resist bacterial colonisation (Bridgett et al., 1992); Dettol® is an established bacteriocidal product. In this study, in addition to the primary aim of examining the in vivo bacterial-release properties of F127 we also seek to consider the implications of combining the two types of treatment by incorporating F127 into the Dettol® formulation.

3.2. Part 1: (i) F127 copolymer (ii) dettol wash, and (iii) a dettol Formulation incorporating F127; effects on skin commensals

The treatments, shown in Table 1(e) and (f), were applied to the skin of the fingers of volunteers. In each case the number of commensal microorganisms retrieved from the surface of the skin was enumerated; the results are presented in Figs. 1 and 2.

Fig. 1 represents the number of skin commensals recovered from a predefined surface area of skin that had been pre-treated with the four formulations described in Table 1. Means are reflective of the mean number of CFU counted on the agar plates for each type of treatment. The results show that the mean number of CFU

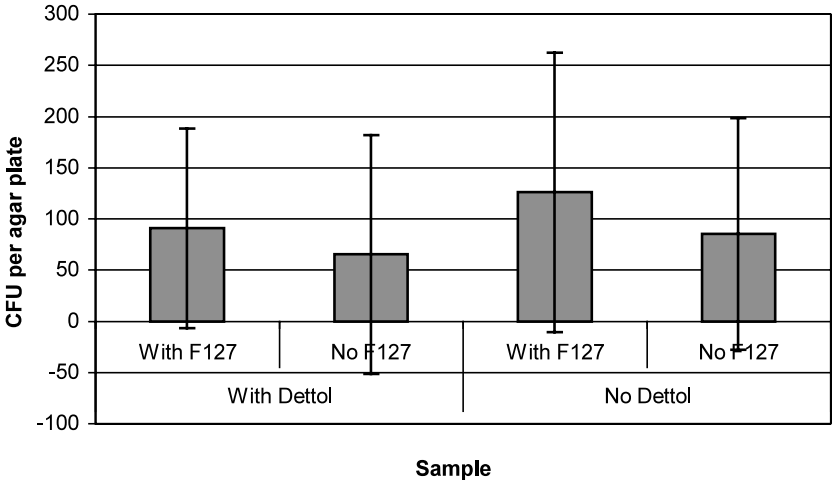

Fig. 1. Number of residual skin commensals (CFU) recovered from the surface of the skin as a function of pre-treatment composition (bars = S.D.).

recovered from skin that had been pre-treated with dettol was slightly lower than that for skin that had been washed with de-ionised water: 78 and 105 CFU per plate, respectively. Conversely, the mean number of organisms recovered from skin pre-treated with F127 was slightly higher than that for untreated skin: 108 and 75 CFU per plate, respectively. These values indicate that F127 may be acting as a very weak bacterial release agent but this effect is too small to be of any clinical significance.

Fig. 2 considers the average numbers of skin commensals recovered from a predefined area of skin surface for each of the six human volunteers as a function of the four types of pre-treatment. As a rule, the use of the bacteriocidal dettol in the pre-treatment formulation results in a reduction of the number of organisms transferred from the skin

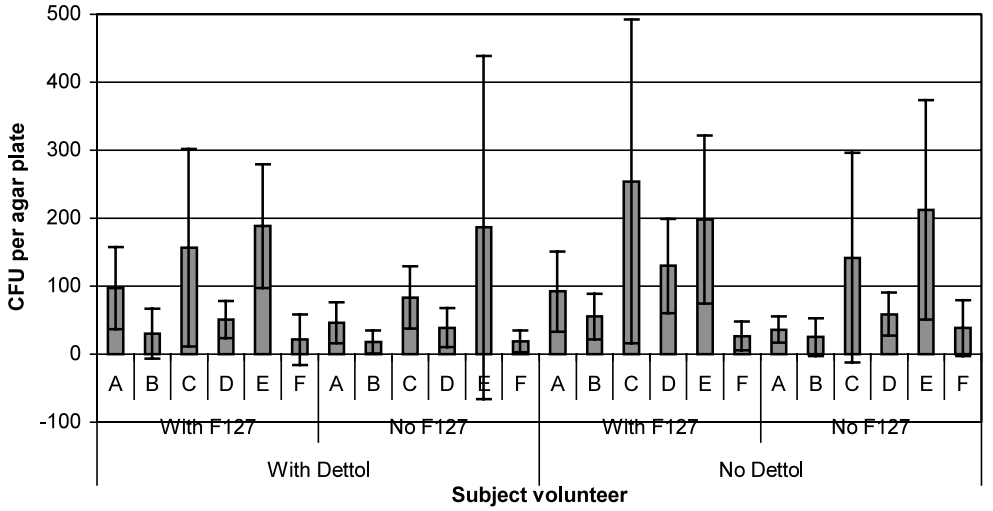

Fig. 2. Variation in number of skin commensals (CFU), recovered from the surface of the skin, as a function of pre-treatment composition; six volunteers (bars = S.D.).

onto agar plates. By contrast, the presence of F127 in the formulation results in a general increase in the number of organisms retrieved from the skin; again highlighting the weak bacterial release properties of this copolymer.

The level 1 analysis 'ANOVA level 1' addresses 'Factors' that are considered to be potential prime variables influencing bacterial attachment, namely: dettol wash; copolymer F127; and the possible co-operative effect of these two components. The second level analysis, 'ANOVA level 2' combines the variables in ANOVA level 1 with possible experimental variables: 'Person', 'Digit' and 'Hand' factor. The analysis was conducted at the 95% confidence level i.e. a 'significant effect' was defined as one with a P-value of <0.05. The results reveal that, for the number of samples under consideration, none of the pre-treatments induced any statistically significant differences in the number of commensals recovered. Variations in skin characteristics and/or residual microflora between individuals may have been factors of greater importance–in terms of number of organisms recovered–than the employment of pre-treatment regimes. Similarly, no statistically significant co-operative effect could be identified for the formulation that incorporates dettol and copolymer F127 (P-value $=0.65$).

In an effort to establish whether any 'treatment effects' were masked by large variations across individual subjects, a second level analysis (ANOVA level 2) was performed. The experimental design allowed an analysis of variance of the following factors: 'Person'; 'Digit'; 'Hand'; and 'Surface Treatment' (copolymer F127 and/or dettol wash). The largest effect apparent from the level 2 analysis was due to the Person factor; reflective of the variations in the amount of skin flora associated with each human volunteer ($P=0.0001$ for Person factor). Once the variation due to the 'Person' factor was removed, statistically significant effects were unmasked for dettol wash, copolymer F127 and Digit factors. In particular, the results showed that dettol wash had a significant effect ($P=0.048$) at reducing bacterial counts. This may be explained in terms of a reduction in the number of organisms present after exposure to the dettol pre-treatment, probably as a result of the antimicrobial action of the wash. In addition, level 2 analysis illustrated that pre-treatment with copolymer F127 (2% solution) induced a significant increase in the numbers of bacteria released from the skin (P-value 0.019). This may have been due to bacteria being detached from the skin through the detergent action of the amphiphilic PEO–PPO copolymer. Although inspection of the data presented in Fig. 2 suggests that a co-operative effect–in terms of bacterial release from skin–may have been present with the combined (dettol wash and copolymer F127) surface treatments, ANOVA-2 analysis showed that this was not of statistical significance ($P=0.808$). Nonetheless, the data need to be considered in the light of the mechanisms of action associated with each pretreatment: dettol, a bacteriocide, reduces the number of live bacteria released onto the agar plate whereas F127, a bacterial release agent, increases the number of cells released.

3.3. Part 2: S. marcescens adsorption assay on human skin pre-treated using (i) F127 copolymer (ii) dettol wash, and (iii) a dettol formulation incorporating F127

The effect of each pre-treatment protocol on the number of *S. marcescens* released on rinsing the skin of the human volunteers and that of the number recovered from the corresponding area of skin (4.91 cm^2) are summarised in Fig. 3. The data from the pre-treatment experiments are presented in two parts: (i) Rinse 1-number of organisms collected on rinsing with de-ionised water, and (ii) Rinse 2-number of organisms collected on subsequent rinsing with a mixture of PBS and Triton X-100.

The use of F127 as pre-treatment results in an increase in the number of organisms detected in Rinse 1; presumably, F127 facilitates the release of organisms by acting as a surfactant. In Rinse 2, the presence of F127 appears to be of little influence in the numbers of organisms recovered. By contrast, the use of dettol wash as a pre-treatment resulted in an increase in the number of organisms collected in Rinse 1 and also an increase in those recovered during Rinse 2.

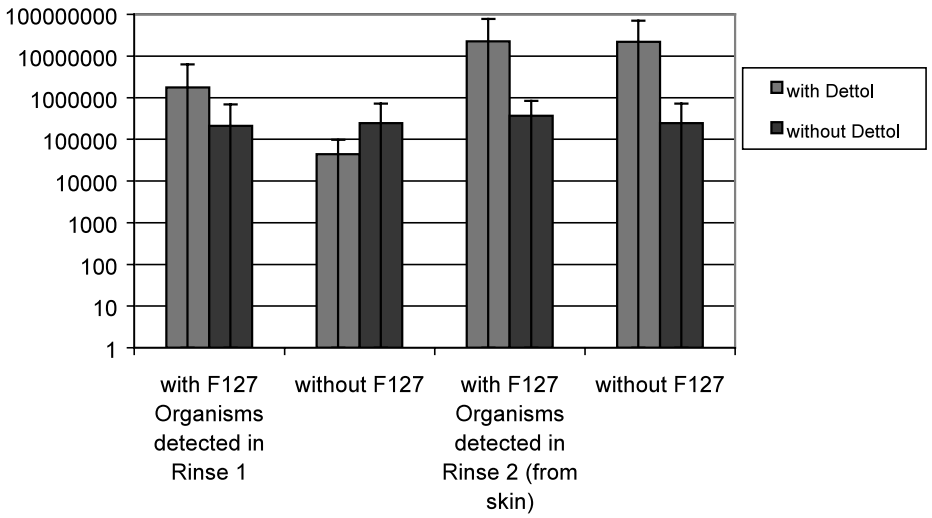

Fig. 3. Comparison of number of organisms collected in Rinse 1 and those recovered during Rinse 2 as a function of the type of pre-treatment (bars = S.D.).

These data were further considered using analysis of variance: in both Rinse 1 and 2, the employment of F127 in the pre-treatment did not appear to induce effects of statistical significance. By contrast, pre-treatment with dettol increased (at the 89.5% confidence level) the number of marker organisms retrieved from the skin (Rinse 2).

3.4. Part 3: adsorption assay on human skin exposed to cultures of S. marcescens and subjected to post-exposure treatment with (i) F127 copolymer (ii) dettol wash, and (iii) a dettol formulation incorporating F127

Cultures of *S. marcescens* were allowed to deposit onto a predetermined area of skin (4.91 cm^2), from human volunteers, and subsequently washed by exposure to vials containing either one of: (i) an aqueous solution of copolymer F127, (ii) dettol wash, or (iii) a dettol formulation incorporating F127. The findings of these, post-treatment, studies are summarised in Fig. 4. The data are presented in two parts: (i) Rinse 1-number of organisms collected in the post-treatment sample vial, and (ii) Rinse 2-number of organisms recovered from the skin (2 ml, in PBS + Triton X-100).

In accordance with expectations, the data show that no viable organisms were present within any of the collection vials that contained the bacteriocidal dettol formulation (Rinse 1). The presence of F127 in the post-treatment formulation appears to have induced a small increase in the number of organisms collected during Rinse 1. At the Rinse 2 stage, Fig. 4, more organisms were collected from the skin when dettol wash was used as the post-treatment. The use of copolymer F127 as a post-treatment appears to have had little effect in the number of bacteria recovered from the skin. The data illustrate that the only statistically significant effect is that due to the presence of dettol in the post-treatment formulation of Rinse 1 ($P = 0.0001$); no statistically significant effects could be identified for Rinse 2 samples.

Overall, this set of experiments appears to support the hypothesis that F127 functions as a weak bacterial-release agent in that solutions containing this copolymer appeared to be more effective at removing organisms from the skin than F127-free formulations. It is worth noting that the incorporation of F127 in the dettol formulation does not appear to impair the efficacy, in terms of bactericidal action, of the commercial product; presumably, the aqueous environment of the formulation suppresses the inherent propensity of

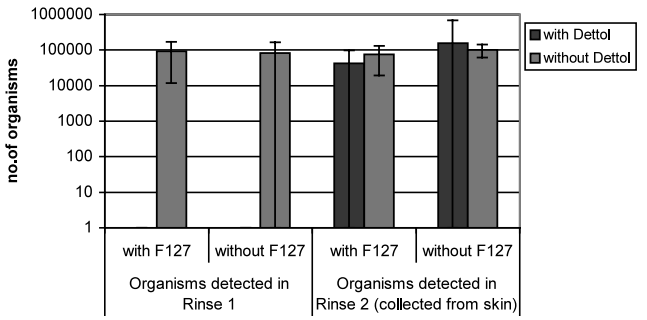

Fig. 4. Comparison of number of organisms collected in Rinse 1 and those recovered during Rinse 2 as a function of the type of post-treatment (bars = S.D.).

para-chloro-*meta*-xylenol to associate, through hydrogen bonded interactions, with the polyether backbone of F127.

4. Conclusions

The results show that, using the methodology employed here, the use of solutions of Pluronic F127 is of little value to formulations designed to decrease bacterial colonisation to human skin. Incorporation of the same co-polymer to a commercially available antibacterial product proved of no significance to the efficacy of that product.

Acknowledgements

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