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## Pluronic<sup>®</sup> F-127 gels incorporating highly purified unsaturated fatty acids for buccal delivery of insulin

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### Abstract

The present study investigated the release profiles of insulin from Pluronic F-127 (PF-127) gel containing unsaturated fatty acids such as oleic acid (18:1), eicosapentaenoic acid (20:5) or docosahexaenoic acid (22:6) and the hypoglycemic effect of insulin following the buccal administration of the gel formulations in normal rats. Insulin release from the gels decreased in the presence of unsaturated fatty acids. Remarkable and continuous hypoglycemia was induced by all PF-127 gels (insulin dose, 25 IU/kg) containing unsaturated fatty acids. PF-127 gels containing oleic acid showed the highest pharmacological availability ( $15.9 \pm 7.9\%$ ). Our findings demonstrate that 20% PF-127 gels containing unsaturated fatty acids are potential formulations for the buccal delivery of insulin. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Insulin; Pluronic F-127 gels; Buccal absorption; Unsaturated fatty acids; Hypoglycemic effect

Various routes are being considered for peptide and protein drug delivery [1]. Of these, the oral mucosa, which comprises the buccal, sublingual, and gingival mucosal, appears to be an alternative route for peptide and protein drug delivery [2]. The attractive features of this mucosa include excellent accessibility, high patient acceptance and

compliance, and significant robustness of the mucosa. In the delivery of insulin via the buccal route of administration, absorption promoters are required in order to obtain satisfactory membrane permeability [2,3]. Recently, classes of polyunsaturated fatty acid absorption enhancers such as eicosapentaenoic (EPA, 20:5 $\omega$ 3) and docosahexaenoic (DHA, 22:6 $\omega$ 3) have been demonstrated to be effective intestinal absorption enhancers of insulin [4,5]. The studies also demon-

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strated that the unsaturated fatty acids did not induce gross damage on the intestinal mucosa. These findings suggest a high possibility that DHA and EPA can be used in a drug delivery system as membrane permeability modifying agents. However, the effects of these polyunsaturated fatty acids on insulin absorption from the buccal mucosa have not been elucidated.

It is quite reasonable that these unsaturated fatty acid enhancers maximize the absorption when locally co-existed with proteins and peptides. In the case of insulin, it is suggested that a certain amount of time was required for insulin molecules to be taken up by the buccal mucosa [3]. Based on this rationale, we prepared insulin gel formulations containing unsaturated fatty acids using Pluronic F-127 (PF-127), a surface-active polyoxyethylene-polyoxypropylene block copolymer with a molecular weight of approximately 12 500 [6]. Their potential usefulness in buccal insulin delivery was evaluated in the present study.

The PF-127 solutions were prepared by the cold method [6]. A weighed amount of PF-127 was slowly added to a cold (5–10°C) pH 7.4 phosphate buffer solution (PBS), with gentle mixing until there was complete dissolution of the polymer. Insulin (porcine insulin, 27.0 IU/mg) was dissolved in 0.1 N HCl, then diluted with PBS and adjusted with 0.1 N NaOH to pH 7.4. The insulin solution was added with gentle stirring to the PF-127 solution. Then, an appropriate amount of DHA (99.0% purity), EPA (99.0% purity) or oleic acid (99.0% purity) was added to the solution with moderate stirring at low temperature (5–10°C) for 20 min. The final concentrations of PF-127 and unsaturated fatty acids were 20 and 5%, respectively. A 20% PF-127 gel formulation containing only insulin was used as a control.

Membraneless dissolution tests were performed for the *in vitro* release studies. The cold PF-127 formulations (3 g) containing insulin (1 mg/g of gel) and fatty acids (5%) were transferred into test tubes and placed in an incubator (37°C). The PF-127 solutions were gelled upon equilibration for 20 min. A 1-ml aliquot of the release medium (pH 7.4, PBS) pre-equilibrated at the experimental

temperature was layered over the surface of the formulations. The test tubes were shaken at 50 strokes/min. At each sampling time, the supernatant was completely removed and replaced with fresh solution in order to maintain sink conditions. The amount of insulin in the released medium was determined as described previously [7].

The spreading radius of exactly 1 ml of the preparation compressed between a square piece of glass and a support plate was measured using a spreadmeter. The preparation sample was put directly on the center of the plate. The measurement was carried out on a support plate at room temperature.

Male Wistar rats weighing 200–220 g, which had fasted for 48 h prior to the experiments, were anesthetized by *i.p.* injection of 50 mg/kg of sodium pentobarbital. The rats were restrained in the supine position on a board that was kept at a surface temperature of 37°C. Buccal dosing was performed after surgical ligation of the esophagus. The trachea was cannulated with polyethylene tubing to allow free breathing. A volume of 0.2 ml of a formulation (insulin dose, 25 IU/kg) was buccally administered to each rat. Blood samples were taken from the jugular vein. The serum glucose level was determined by using a Glucose B-test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The average predose serum glucose levels of the animals used in the study was  $104.1 \pm 15.0$  mg/dl (range = 79.9–122.4 mg/dl). Post-dose levels of the serum glucose were expressed as a percentage of the predose level. The areas below the baseline levels in the percentage change versus time curves for 0–8 h was obtained and used for calculation of pharmacological availability. The pharmacological availability was calculated relative to that administered by the subcutaneous route using the methods described in Ref. [8]. The experimental procedures described above were performed according to the rules set by the Committee on Ethics in the Care and Use of Laboratory Animals in Hoshi University.

Each value was expressed as the mean  $\pm$  S.D. For group comparisons, the one-way layout ANOVA with duplication was applied. Significant differences in the mean values were evaluated by Student's unpaired *t*-test.

The property of reversal thermal gelation exhibited by PF-127 aqueous solutions in the 20–35% concentration range has been used for various drug delivery systems [9,10]. Another important characteristic of PF-127 gels is the enhancement of the stability of proteins loaded into the gel matrix [11]. In order to maximize its absorption enhancement effect, unsaturated fatty acid is required to be co-dissolved into the same preparation with insulin [7]. From this point of view, PF-127 is thought to be a suitable vehicle for preparing insulin formulations co-dissolved with unsaturated fatty acid.

The *in vitro* release of insulin from the PF-127 gel formulations is shown in Fig. 1. The results of the *in vitro* study indicated that the presence of fatty acids in PF-127 gels decreased the insulin release. The results suggested that the presence of fatty acids in PF-127 gels may interfere with the release of insulin from the formulations. The PF-127 gels forms two distinct amphiphilic regions after hydration [12]. The partition of drugs between these two segments of the polymer network is known to influence the diffusion and release of drugs from PF-127 gels. It has been reported that the incorporation of hydrophilic additives into PF-127 gel formulation accelerated the release of

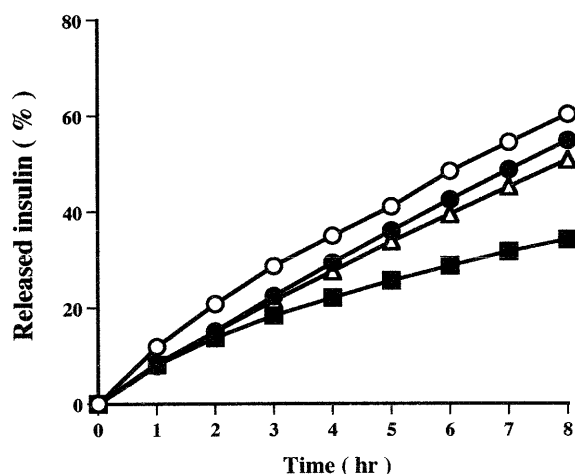


Fig. 1. Release profiles of insulin from various PF-127 gel formulations. PF-127 gel without fatty acid (○), containing oleic acid (●), EPA (△) and DHA (■). Each value represents the mean  $\pm$  S.D. ( $N = 3$ ).

Table 1

Spreadability of pluronic F-127 gel formulations incorporating unsaturated fatty acid<sup>a</sup>

Preparation	Radius (mm)
Pluronic F-127 gels	1.68 $\pm$ 0.05
Pluronic F-127 gels incorporating Oleic acid	1.35 $\pm$ 0.14
Pluronic F-127 gels incorporating EPA	1.40 $\pm$ 0.09
Pluronic F-127 gels incorporating DHA	1.33 $\pm$ 0.18

<sup>a</sup> Each value represents the mean  $\pm$  S.D. ( $N = 4-5$ ).

\*Statistically significant difference between the two formulations ( $P < 0.01$ ).

the drug [13]. On the other hand, less hydrophilic additives decreased the rate of drug release from it. The mechanism of the reduced release rate of the hydrophilic drug in the presence of hydrophobic additives may be due in part to reductions in the numbers and dimensions of the aqueous channels through which hydrophilic solutes diffuse.

Another factor affecting drug release may be the viscosity of the formulations. Rheological parameters of PF-127 gel formulation containing fatty acids are shown in Table 1. All formulations containing fatty acids exhibited significantly lower spreadability than the control PF-127 formulation. The increased viscosity might have contributed, in part, to the decreased rate of insulin release.

Fig. 2 shows the changes in serum glucose levels following buccal administration of insulin-loaded PF-127 gels on serum glucose levels. As shown in Fig. 2, buccal administration of insulin formulations results in a negligible absorption without unsaturated fatty acids. Rather, the intrinsic blood glucose levels rose continuously due to surgical stress. On the other hand, significant reduction of the serum glucose levels to all formulations containing unsaturated fatty acids was observed. The hypoglycemic effects were continued and the blood glucose level of the formulation containing oleic acid did not reach pre-dose level during the study period.

The pharmacological availabilities calculated from the buccal absorption study are shown in

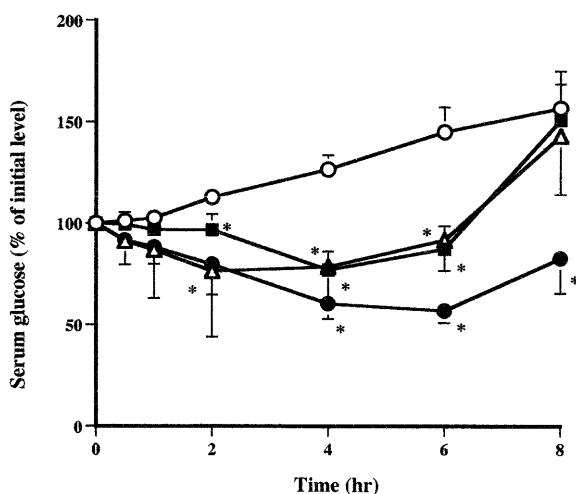


Fig. 2. Effect of intra-buccal administration of insulin-loaded PF-127 gel formulations on serum glucose levels. PF-127 gel without fatty acid (○), containing oleic acid (●), EPA (△) and DHA (■). Each value represents the mean  $\pm$  S.D. ( $N=4$ ). Comparisons calculated at each period PF-127 gel without fatty acid vs. PF-127 gel containing oleic acid, EPA or DHA ( $*P < 0.01$ ).

Table 2. The PF-127 gels containing oleic acid, which showed rapid insulin release, exhibited the highest relative hypoglycemic efficacy relative to subcutaneous administration. On the other hand, PF-127 gels containing DHA, which had the smallest insulin release rate, showed a small hypoglycemic effect in the initial phase of administration study and a low pharmacological availability value. These results are not consistent with those obtained by our previous rectal administration

Table 2  
Pharmacological availability<sup>a</sup>

Preparation	F (%)
Pluronic F-127 gels	1.0 $\pm$ 0.01
Pluronic F-127 gels incorporating Oleic acid	15.9 $\pm$ 7.9
Pluronic F-127 gels incorporating EPA	3.4 $\pm$ 1.2
Pluronic F-127 gels incorporating DHA	4.1 $\pm$ 3.4

<sup>a</sup> Each value represents the mean  $\pm$  S.D. ( $N=4$ ).  
\*Statistically significant difference between the two formulations ( $P < 0.05$ ).

study [7]. In the study, no significant differences could be observed among the relative hypoglycemic efficiencies of PF-127 gels containing the different unsaturated fatty acids. The absorption of insulin from the gels occurred rapidly in the rectum. In contrast, the buccal mucosa would offer sustained release profile for buccally-administered drugs due to its depot function. It has been suggested that longer indwelling of the peptide drug in the mucosa can make the peptide drug more susceptible to the proteolytic enzymes, which exist even in the buccal membrane [14]. Therefore, the differences in the insulin release rate among the formulations may lead to differences in the pharmacological availability of the buccal administration.

Unsaturated fatty acids used in this study were known as strong but highly safe membrane-modifying agents [4,5]. The ability of insulin free or associated with unsaturated fatty acids, to diffuse through the buccal mucosa in normal rats is an important finding in the search and development of a mucosal dosage form for insulin or any other peptide drugs.

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