COSMETIC PATCHES CONTAINING SOY ISOFLAVONES

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Introduction

Phytoestrogens are plant-derived nonsteroidal compounds that possess estrogen-like biological activity. Among all classes of phytoestrogens that have been reported, lignans, coumenstans, and isoflavones appear of particular interest from a health perspective and in particular genistein (GEN) and daidzein (DAI). These naturally occurring isoflavones derived from soybeans have shown estrogenic activity. Epidemiological studies suggest that an elevated intake of DAI and GEN reduced the incidence of many hormone-dependent diseases. Researchers demonstrated that such isoflavones reduce the risk of breast and prostate cancer, as well as cardiovascular diseases, and improve bone health in an animal model. Moreover, they have been proposed as an alternative to conventional hormone replacement therapy for the treatment of postmenopausal diseases.
As cosmetic use, phytoestrogens and in particular DAI and GEN are of interest as they can intensely moisturize and restructure the skin. The skin's surface regain density and skin features are visibly toned.
Till today the main intake of soy derivatives is in dietary or as supplements. Transdermal application can be considered as an alternative route of administration of isoflavones, that allows to avoid the metabolic processes associated with the gastrointestinal system and liver. Moreover, by using patches, it could be obtained a prolonged release of GEN and DAI.
The aim of this study was to evaluate the feasibility of the (trans)dermal administration of GEN and DAI contained in a commercial standardized soy dry extract by using monolayer methacrylic patches. The influence of the polymer type and enhancers on GEN and DAI skin permeation was evaluated. Preliminarily the effects induced by different skin permeation enhancers on GEN and DAI skin permeation were verified. The skin permeation study was performed by using modified Franz diffusion cell and the human stratum corneum and epidermis as a membrane.

MATERIALS and METHODS

Materials

Genimax, soy dry extract, containing 40% w/w of total isoflavones, corresponding to 25% w/w of GEN and DAI (Sochim, I).

Transcutol, TR, and Labrasol, LB (Gattefossè, F). Propylene glycol, PG (Carlo Erba, I). Glycerol, GLY, and polyethylene glycol 400, PEG400 (ACEF, I). Oleic acid, OA (Polichimica, I). Eudragit® E 100, EUE100, and Eudragit® L 100-55, EUL100 (Röhm, G). Kollidon 90F, K90F, (BASF, G). Lauric acid, LA, (Fluka, G). Polyethylene was used as backing layer.

Solubility studies

The solubility of the extract in water and in different permeation enhancers was obtained by equilibrating for 24 hours large excesses of the solute and the vehicle at 32°C. The permeation enhancers were: OA, LB, TR, PEG400 and PG.

Patch preparation

The patches were prepared by using a laboratory coating unit Mathis LTE-S (M) (CH). The composition of the patches is reported in Table 1.

Table 1: Composition of the patches containing soy dry extract (% w/w)

Formulation	Soy dry extract	EUE100	EUL100 + KOH	K90F	GLY	PEG400	PG	LA
1	13.5	-	29.7	19.6	17.2	-	20.0	-
2	13.5	-	29.7	19.6	17.2	20.0	-	-
3	13.5	30.3	-	31.1	-	-	20.1	5.0
4	13.5	30.3	-	31.1	-	20,1	-	5.0

GEN and DAI content

A 2,54 cm^2 sample of the patch was solubilized with methanol. The solutions were filtered and assayed by the HPLC method described below.

Ex vivo permeation study

The skin permeation tests were performed by using modified Franz diffusion cells and as a membrane human stratum corneum and epidermis. Sink conditions were maintained throughout the experiment. After 24 h solutions or the patch were removed and the epidermal surface of the skin was washed, then cut into small pieces and added to methanol. The suspension, soaked for 30 min, was stored for 12 h and then filtered. The GEN and DAI were assayed by the method described below.

Adhesion properties

Peel adhesion 180° test. The force was expressed in centiNewton (cN) per centimeter width of patch under test. Peel adhesion values were obtained from triplicate determinations.

Probe tack test. The force was expressed in Newton (N). Tack values were obtained from five determinations.

GEN and DAI assay

The analytical instrument was an HPLC (HP 1100, Chemstations, Hewlett Packard, USA). Conditions of the isocratic method are injection volume at 10 μl, at a flow rate of 1 ml/min, and ultraviolet absorbance was monitored at 260 nm. Separation of the individual isoflavones was obtained on a C18 reverse-phase column (Hypersil, 5 μmSpherisorb ODS2, 4.6× 200 mm, Waters, UK). The mobile phase was methanol-2 mmol/L and ammonium acetate (55/45, v/v) and temperature was fixed at 25◦C. A standard calibration curve (0.1–50 μg/ml) for each molecule was used. The detection limit was 0.03 μg/ml for DAI and 0.05 μg/ml for GEN. Resolution from the background noise was adequate at this level. The other substances of the soy extract did not interfere in DAI and GEN determination. The retention time of GEN and DAI was 10 min and 7 min, respectively.

Conditions of the gradient method were injection volume at 10 μl, at a flow rate of 1 ml/min, and ultraviolet absorbance was monitored at 260 nm. Separation of the individual isoflavones was obtained on a C18 reverse-phase column (Hypersil). Temperature was fixed at 25◦C. The mobile phase was 100% of 10 mmol/L ammonium acetate (0.1% trifluoroacetic acid) held isocratic for the first 2 min and then decreased to 50% at a constant gradient from 2 to 24 min, then finally held isocratic with 50% acetonitrile and 50% 10 mmol/L ammonium acetate (0.1% trifluoroacetic acid) for 5 min, before being returned to the original composition of 100% 10 mmol/L ammonium acetate (0.1% trifluoroacetic acid). The retention time of GEN and DAI was 25 min and 22 min, respectively.

RESULTS and DISCUSSION

Characterization of the extract

The dry soy extract, analyzed by the gradient method, exclusively contained the aglycones GEN and DAI in the amount of 24% m/m, corresponding to 11.7% m/m GEN and 12.3% m/m DAI and indicating that the extraction method caused the hydrolysis of the conjugated forms of the isoflavones.

GEN and DAI solubility

The solubilities of GEN and DAI contained in the dry extract in the selected vehicles are reported in Table 2. The solubility of both the isoflavones in water was the lowest and very close to that measured in OA (less than 0.1 mg/ml, Table 2). GEN was more soluble than DAI in PG and LB.

Table 2 - Solubility of GEN and DAI contained in the dry soy extract (n=3, mean ± s.d.)

Vehicle	GEN (mg/ml)	DAI (mg/ml)
PEG400	8.51 ± 0.43	8.53 ± 0.46
LB	8.05 ± 0.21	5.73 ± 0.12
TR	6.83 ± 0.03	6.86 ± 0.03
PG	4.34 ± 0.03	2.61 ± 0.02
OA	0.025 ± 0.008	0.001 ± 0.001
Water	0.015 ± 0.005	0.013 ± 0.01

GEN and DAI content

In all patches the average content of GEN was 107,507±3,422 μg/cm^2±d.s. and of DAI was 21,327±1,095 μg/cm^2±d.s.

Ex vivo skin permeation of phytocomplex and patch

PEG400 induced the highest fluxes. These flux values were not significantly different than those obtained by using PG solutions. The fluxes obtained by using the other enhancers were less than 0.04 μg/cm^2/h (Table 3).

Table 3 - Permeation parameters of GEN and DAI from the dry soy extract saturated vehicles (n=3, mean ± s.d.)

	GEN			DAI		
Vehicle	**Permeated amount after 24h (μg/cm^2)**	**Flux (μg/cm^2/h)**	**K_p (cm^2/h)**	**Permeated amount after 24h (μg/cm^2)**	**Flux (μg/cm^2/h)**	**K_p (cm^2/h)**
PEG400	31.7 ± 8.7	1.3 ± 0.4	1.5 x 10^{-4}	23.9 ± 11.9	1.0 ± 0.5	1.2 x 10^{-4}
LB	5.6 ± 0.4	-**	-	-*	-	-
TR	5.8 ± 0.2	-**	-	-*	-	-
PG	12.3 ± 3.2	0.5 ± 0.1	1.2 x 10^{-5}	9.8 ± 3.9	0.4 ± 0.2	1.5 x 10^{-5}
OA	-*	-	-	-*	-	-
Water	-*	-	-	-*	-	-

Note: *not detectable; **lower than 0.1 μg/cm^2/h.

Considering the lipophilic characteristic of these molecules (logP GEN: 3,72; logP DAI: 4,72) they could be retained in the skin in a significant amount. Therefore, in the case of PEG, the permeation enhancer showing the highest permeated amount of both isoflavones, the retained amount into the stratum corneum and epidermis was measured: GEN 38.9 mg/cm^2 and DAI 2.8 mg/cm^2.

PEG400 and PG were therefore used as enhancers in the patches. For each patch the permeated amounts of GEN were higher than those of DAI. In the case of GEN, the patches based on EUL100 showed higher permeated and retained amount than those obtained by using EUE100 (Table 4). No differences in term of skin permeation seemed to be due to the type of enhancer (Table 4).

Table 4 - Permeation parameters of GEN and DAI from patches (n=3, mean ± s.d.)

	GEN			DAI		
Formulation	**Permeated amount after 72h (μg/cm^2)**	**Flux (μg/cm^2/h)**	**Retained amount (μg/cm^2)**	**Permeated amount after 72h (μg/cm^2)**	**Flux (μg/cm^2/h)**	**Retained amount (μg/cm^2)**
1	2,0±0,1	0,026±0,017	17,4±5,8	0,4±0,3	0,004±0,003	2,1±2,1
2	1,7±0,7	0,021±0,001	11,9±6,7	0,6±0,2	0,005±0,004	6,3±4,0
3	0,4±0,7	0,002±0,001	5,6±4,3	0,6±0,3	0,005±0,002	0,6±0,2
4	0,5±0,0	0,005±0,001	6,5±5,7	0,6±0,3	0,006±0,005	3,1±3,1

Adhesion properties

The peel adhesion values of patches based on EUL100 were higher than those of patches based on EUE100. Formulations containing PEG400 showed peel adhesion and tack values slightly higher than those of formulations containing PG (Table 5). The CV of each sample in the peel adhesion assay was less than 10% indicating a good matrix cohesion.

Table 5 - Peel adhesion and tack values of patches

Formulation	**Peel adhesion (cN/cm; n=3, mean±d.s.)**	**Probe Tack Test**	
		Max detachment load (N; n=5, mean±d.s.)	**Detachment work (mJ; n=5, mean±d.s.)**
1	137±3	0,23±0,08	0,094±0,036
2	151±55	0,27±0,06	0,097±0,013
3	82±5	1,04±0,31	0,538±0,165
4	93±1	1,23±0,09	0,656±0,034

CONCLUSIONS

Monolayer transdermal patches containing soy extract with good technological characteristics were prepared with matrices made of methacrylic copolymers.

Best performances in terms of retained amount into skin were obtained by using EUL100 as polymeric system. PEG400 and PG, the selected enhancers, seems to not have a different enhancement effect.