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# Skin penetration of flavonoids - influence of nonionic surfactants

Keywords: Flavonoids, nonionic surfactants, polyoxyethylene alkyl ethers, lipophilic membrane, skin permeation

## Synopsis

Flavonoids are polyphenolic substances widely distributed in the nature in the form of glycosides and aglycones. The growing interest in the application of these compounds in cosmetology is connected with their beneficial biochemical properties. To influence the skin effectively, the molecule of flavonoid has to overcome the skin barrier which is *stratum corneum*.

The studies introduced in the article are carried out employing side-by-side diffusion cell and lipophilic model membrane mounted in. The permeation of quercetin, rutin, catechin and flavonoids from grape leaf extract has been determined and permeation coefficients were calculated. The influence of nonionic surfactants present in many cosmetic formulations next to flavonoids has been described.

To elucidate the mechanism of hampering effect of nonionic surface active agents on permeation rate of flavonoids the partition coefficients in the octanol/water extraction system and solubility of flavonoids in the presence and absence of surfactant have been determined. Correlations between permeation coefficients of flavonoids, their solubility and log P have been introduced.

## Introduction

The very various group belonging to polyphenols are flavonoids, compounds occuring in the plant kingdom both in glycoside and in aglycone form [1]. The scientific approaches report that these molecules display beneficial influence on human body, including skin [2,3]. The general formula of flavonoid is composed of two benzene rings linked by a carbon chain (Fig. 1).

The skin is the first line of our defence against external agents. This organ plays a role of very effective barrier for compounds that have been applied onto its surface and are supposed to penetrate it [7]. In the skin flavonoids could play a very important role of free radical scavengers and anti-oxidising agents in the most upper layer of the skin- *stratum corneum* [8]. Penetrating deeper, to the level of epidermis - flavonoids are involved in many enzymatic reactions [9]. The dee-



**Figure. 1** The general structure of flavonoids
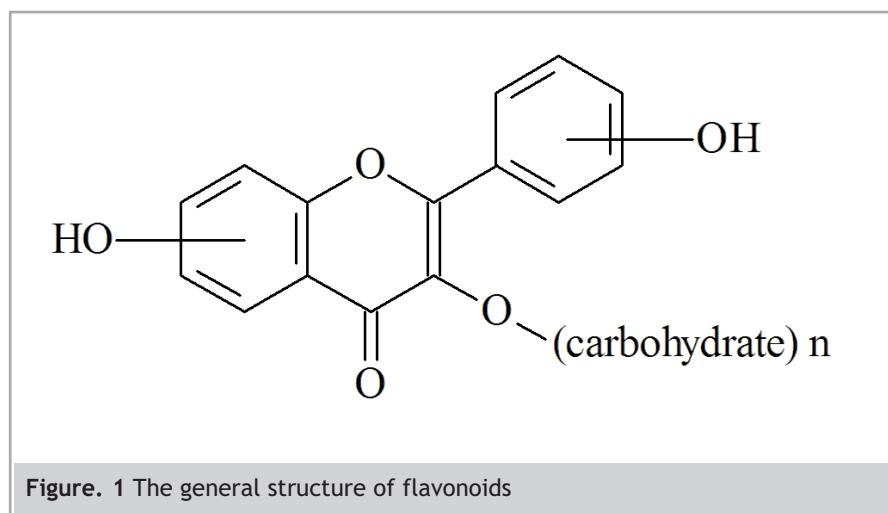
The surprising diversity of flavonoids is a consequence of their chemical heterogeneity. Flavonoids have been recognised as compounds possessing significant influence on human organism including skin.

These substances display strong antioxidant, free-radical-scavenging and metal-chelating activity as well as many other activities [4,5]. It has been proven, that the process of lipid peroxidation can be effectively inhibited by flavonoids [6].

pest skin layer, *dermis* could be protected by flavonoids as well, mainly by influencing the permeability and fragility of microvessels [10,11]. All of these beneficial effects of flavonoids on human skin can exist only if one condition is fulfilled – the flavonoid molecule is able to overcome the skin barrier which is *stratum corneum*.

It is worth noticing, that permeation profile of flavonoids depends also on the presence of other substances present in the system [12]. One of the most com-

mon substances present in the cosmetic formulations are nonionc surfactants [13]. It has been documented that these compounds can play a role of skin penetration modifiers [14,15]. The studies have shown that non-ionic surfactants can interact with the skin, either by penetration into the horny layer or by extraction of intercellular lipids. At the same time the presence of surfactant in the donor solution can decrease the activity of permeant by solubilisation process – the substance which is anchored to the micelles remains in the vehicle [16,17,18].

To determine the influence of selected non-ionic surface active agents on the permeation profile of flavonoids the model approach has been adopted. The side-by-side diffusion cell has been employed and the lipophilic membrane mounted in [19,20].

The very common flavonoids: rutin, quercetin and catechin have been used as model substances (Fig. 2).

Having in the view the fact that flavonoids are present in the cosmetics mainly in the form of natural mixtures the permeation rate of flavonoids from grape leaf extract has been determined.

All experiments were carried out using phosphate buffer (pH=7.4) as a solvent what ensured constant pH. Three surface active agents have been selected: polyoxyethylene 12 cetostearyl ether, polyoxyethylene 20 cetostearyl ether and polyoxyethylene 30 cetostearyl ether (Fig. 3).

The permeation profile of flavonoids in

$$R\text{-}(OCH_2CH_2)_nOH \qquad R= \text{blend of cetyl and stearyl radicals}$$
$$n = 12, 20 \text{ or } 30$$

**Figure. 3** Schematic composition of polyoxyethylene cetostearyl ethers

the presence of these common cosmetic ingredients has been plotted and influence of oxyethylene (EO) chain length in the surfactant on migration process of flavonoids was investigated.

■ Materials and methods

**Materials**
Polyoxyethylene 12 cetostearyl ether, polyoxyethylene 20 cetostearyl ether and polyoxyethylene 30 cetostearyl ether (Eumulgin B1, Eumulgin B2 and Eumulgin B3 respectively) were obtained from Cognis company. Catechin was purchased from Fluka, rutin and quercetin from Sigma – Aldrich. The grape leaf extract was supplied by Croda. Polyester membrane was purchased from The Institute of Chemistry and Nuclear Technique, Warsaw. Magnetic stirrer ES 24, 1000rpm was obtained from Conbest. To determine the concentration of investigated flavonoids the spectrophotometer Hitachi 3300 has been employed.

**Preparation of lipid membrane and permeation process**
The polyester micro-filter membrane of 12mm radius, 12 micrometers thickness and 0,4 micrometer diameter of pores has been employed to sandwich liposo-

mes composed of the stratum corneum lipids in between. After spreading the lipids on polyester the membrane was dried at the room temperature for 24 hours, mounted in a side-by side diffusion cell and stabilized in the phosphate buffer (pH= 7.4).
The experiments were carried out employing side-by-side diffusion cell (volume of one half-cell – 26 ml). The diffusion area of the membrane is 1.77 cm$^2$.
The donor cell was filled with 50 µg/ml solution flavonoid in phosphate buffer (pH=7.4). Fresh phosphate buffer was placed in acceptor cell and samples were taken during 72 hours.
Concentration of flavonoids has been determined employing spectrophotometric analytical methods. Quercetin was determined at 375 nm, rutin at 365 nm and catechin at 420 nm. The absorbance of flavonoids from the grape leaf was measured at 275 nm.

Determination of permeability and apparent partition coefficients
The permeation abilities of investigated flavonoids have been introduced in the form of permeability coefficients. The cumulative amount flavonoids was plotted against time and the steady-state was determined to calculate flux [21]. The re-
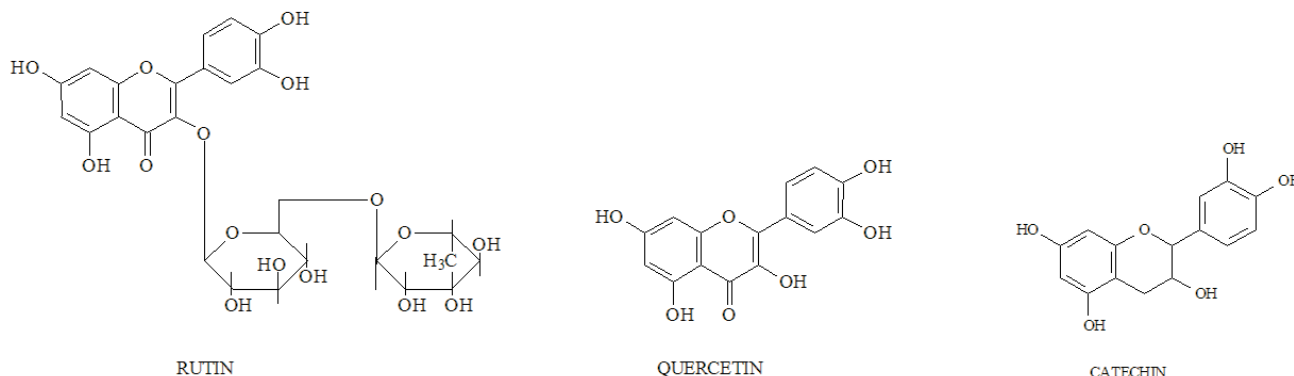


RUTIN          QUERCETIN          CATECHIN

**Figure. 2** Structures of selected flavonoids

sults have been presented in the form of permeability coefficient (Kp).

$$KP = \frac{J}{C_v} \qquad (1)$$

where:
J - flux at steady-state, $C_v$ - concentration of the substance in the donor solution at the beginning of the experiment.

To determine apparent octanol/water partition coefficients of flavonoids in the presence and absence of nonionic surfactants the shake-flask method has been employed [22].
Octanol and water were saturated with each other and investigated substance was dissolved in water. 6% of nonionic surfactant was incorporated to the water phase. Both phases have been collected and shaken for 4 hours. The partition coefficient was calculated from equation 2 and denoted as logP.

$$P = C_o/C_w \qquad (2)$$

$C_o$ in the equation 2 refers to the concentration of the substance in octanol phase, $C_w$ - to the flavonoid concentration in water phase.

## ■ Results and discussion

**Polyoxyethylene alkyl ethers and their effect on permeation process of flavonoids**

Polyoxyethylene alkyl ethers are very common surfactants that are incorporated to the cosmetic formulations. The skin penetration process of flavonoid molecule which is added to the same system can be influenced by the presence of non-ionic surfactant.
The first part of our study aimed at determination of migration rate of flavonoids from natural mixture – grape leaf extract. The influence of three selected polyoxyethylene cetostearyl ethers of different EO chain length on the permeability coefficient of flavonoids from grape leaf has been investigated (Table I).
Analysing the permeation ability of flavonoids from the natural mixture in the presence of polyoxyethylene cetostearyl ethers one can notice that all investigated non-ionic surfactants hamper the migration rate of flavonoids. The most effective permeation inhibitor is polyoxyethylene 30 cetostearyl ether.
Further part of the research comprised the determination of non-ionic surfactant influence on the permeation profile of pure flavonoids – quercetin, catechin and rutin. Results have been presented in the table II.
The studies carried out for pure flavono-ids confirmed results obtained for natural mixture – grape leaf extract. It can be observed that all examined surfactants slow dawn the permeation rate of all flavonoids.

To enable the comparison of the surface active agents influence on the permeation profile of quercetin, catechin and rutin the results from Table II have been introduced in the form of ΔKp (Table III). The most effective hampering effect is displayed by polyoxyethylene 30 cetos-

| EO chain length | $Kp * 10^7$ $[cm\ s^{-1}]$ | $\Delta Kp * 10^7$ $[cm\ s^{-1}]$ |
|---|---|---|
| control | $1.44 \pm 0.19$ | - |
| 12 | $1.30 \pm 0.17$ | $-0.14 \pm 0.19$ |
| 20 | $0.74 \pm 0.09$ | $-0.70 \pm 0.19$ |
| 30 | $\mathbf{0.34 \pm 0.04}$ | $\mathbf{-1.10 \pm 0.19}$ |

Where Kp – permeability coefficient

ΔKp = Kp in the presence of surfactant – Kp in the absence of surfactant

control – system without surfactant

**Table I** Permeability coefficients of flavonoids from grape leaf in the presence of polyoxyethylene cetostearyl ethers (mean of 6 experiments ± S.D.)

| EO chain length | $Kp_Q * 10^6$ $[cm\ s^{-1}]$ | $Kp_C * 10^6$ $[cm\ s^{-1}]$ | $Kp_R * 10^7$ $[cm\ s^{-1}]$ |
|---|---|---|---|
| control | $20.80 \pm 2.01$ | $2.86 \pm 0.18$ | $6.11 \pm 0.37$ |
| 12 | $17.23 \pm 1.67$ | $0.45 \pm 0.03$ | $1.31 \pm 0.08$ |
| 20 | $16.41 \pm 1.59$ | $0.39 \pm 0.02$ | $0.84 \pm 0.05$ |
| 30 | $2.03 \pm 0.19$ | $0.64 \pm 0.04$ | $0.77 \pm 0.05$ |

where $Kp_Q$, $Kp_C$ and $Kp_R$ – permeability coefficients for quercetin, catechin and rutin respectively.

**Table II** Permeability coefficients of flavonoids in the presence of polyoxyethylene cetostearyl ethers (mean of 6 experiments ± S.D.)

| EO chain length | $\Delta Kp_Q * 10^6$ $[cm\ s^{-1}]$ | $\Delta Kp_C * 10^6$ $[cm\ s^{-1}]$ | $\Delta Kp_R * 10^7$ $[cm\ s^{-1}]$ |
|---|---|---|---|
| control | - | - | - |
| 12 | $-3.57 \pm 2.01$ | $-2.41 \pm 0.18$ | $-4.80 \pm 0.37$ |
| 20 | $-4.39 \pm 2.01$ | $\mathbf{-2.47 \pm 0.18}$ | $-5.27 \pm 0.37$ |
| 30 | $\mathbf{-18.77 \pm 2.01}$ | $-2.22 \pm 0.18$ | $\mathbf{-5.34 \pm 0.37}$ |

Where ΔKp = Kp in the presence of surfactant – Kp in the absence of surfactant.

**Table III** Influence of polyoxyethylene cetostearyl ethers on the permeability coefficients of flavonoids (mean of 6 experiments ± S.D.)

tearyl ether in the case of quercetin and rutin. Polyoxyethylene 20 cetostearyl ether tangibly decreases the permeation coefficient of catechin. The inhibiting influence of polyoxyethylene cetostearyl ethers increases with increasing length of oxyethylene chain.

*The influence of non-ionic surfactant concentration*

It is worth noticing that composition of cosmetic formulation can change after application on the skin surface. The main reason of such a behavior is evaporation of water from the system. According to the theory introduced above, the concentration of nonionic surfactant contained in the cosmetic increases after application.

The second stage of our research concerned the influence concentration of polyoxyethylene 20 cetostearyl ether on the permeation profile of flavonoids. The effect of this study for quercetin and catechin has been presented on the diagram (Fig.4).

The experiment has proven that hampering effect of examined non-ionic surfactant increases with increasing concentration of this substance. Less influence of polyoxyethylene 20 cetostearyl ether on the migration rate can be observed for catechin. In the case of quercetin the highest decrease in the permeation coefficient appears in the range of 6-12% of surface active agent. Catechin is the most affected when the concentration of surfactant is 0.1-1%. Similar investigation was carried out for rutin and flavonoids from grape leaf (Fig.5)

The migration rate of rutin slow down with increasing concentration of polyoxyethylene 20 cetostearyl ether and character of the slope is very strongly decreasing in the range of 1-3% of surfactant.
The curve plotted for flavonoids from grape leaf is more irregular displaying slight increase in the range of 1-3% of surface active agent.
To elucidate the hampering effect of examined surfactants on permeation of
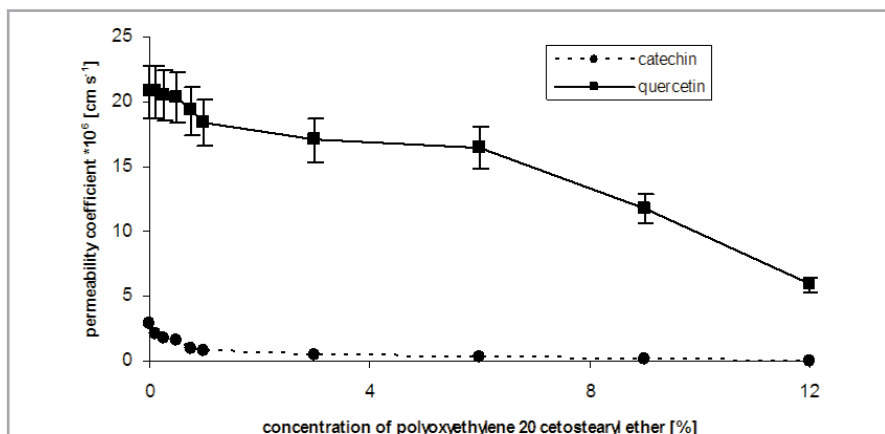


**Figure. 4** Permeability of quercetin and catechin as a function of polyoxyethylene 20 cetostearyl ether concentration (mean of 6 experiments).
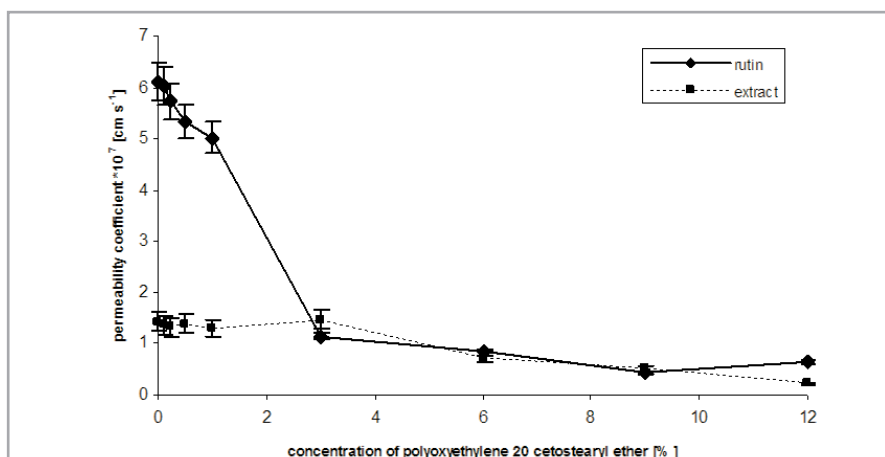


**Figure. 5** Permeability of rutin and flavonoids from grape leaf extract as a function of polyoxyethylene 20 cetostearyl ether concentration (mean of 6 experiments).
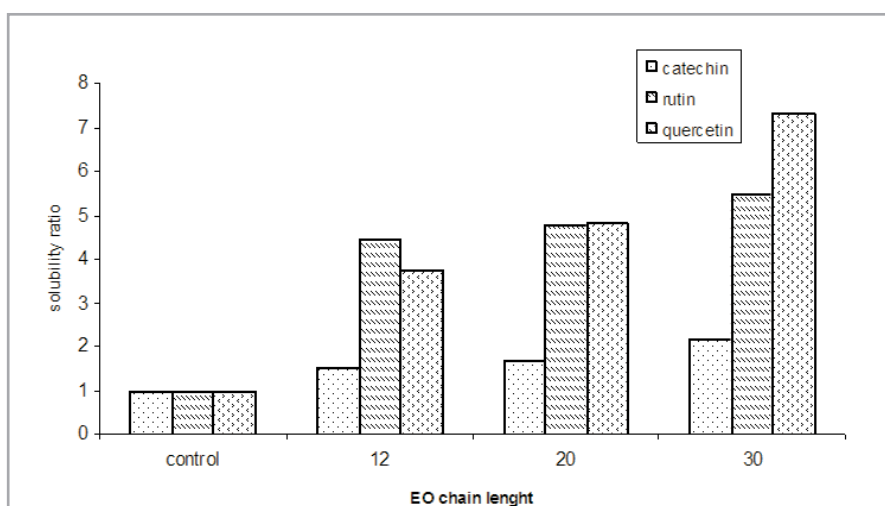


**Figure. 6** Solubility of flavonoids in the presence of polyoxyethylene cetostearyl ethers where solubility ratio (SR) is defined as follows:

$$SR = \frac{\text{flavonoid solubility in phosphate buffer containing hydrophilic substance}}{\text{flavonoid solubility in phosphate buffer}}$$

flavonoids the influence of these substances on solubility and octanol/water partition coefficient of flavonoids was investigated.

## Polyoxyethylene alkyl ethers and their effect on solubility of flavonoids.

One of the most important factor in the permeation process is activity of the permeant in donor solution. Activity of the substance can be expressed by the ratio of concentration in the donor solution to solubility of the substance. This means that activity is inversely proportional to the solubility of the permeant. Thus, the increase in the solubility causes the decrease in activity of the substance what results in the reduction of driving force for the permeaton process.

The next stage of the study aimed at determination of non-ionic surfactant influence (6%) on the solubility (and activity at the same time) of flavonoid in the donor solution (Fig. 6)

Discerning analysis of presented results allows for noticing that introduction of surface active agent to the system increases solubility of all examined flavonoids. The most significant increase in the solubility can be observed for quercetin and rutin, solubility of catechin is affected in much less extent. In all cases the solubility of the substance increases with increasing EO chain length of the surfactant.

The analysis of the diagrams presented in the paper allowed us to plot correlations between permeability coefficients and solubility of flavonoids (Fig. 7).

Correlation reported at the Fig. 7 displays the relation between migration rate of rutin in the presence of particular non-ionic surfactants and solubility ratio of these flavonoids. It can be easily observed that permeability coefficients of rutin decrease with increasing value of solubility ratio. Hampering effect of polyoxyethylene cetostearyl ethers increases with increasing length of EO chain. Similar relation can be plotted for quercetin (Fig.8).
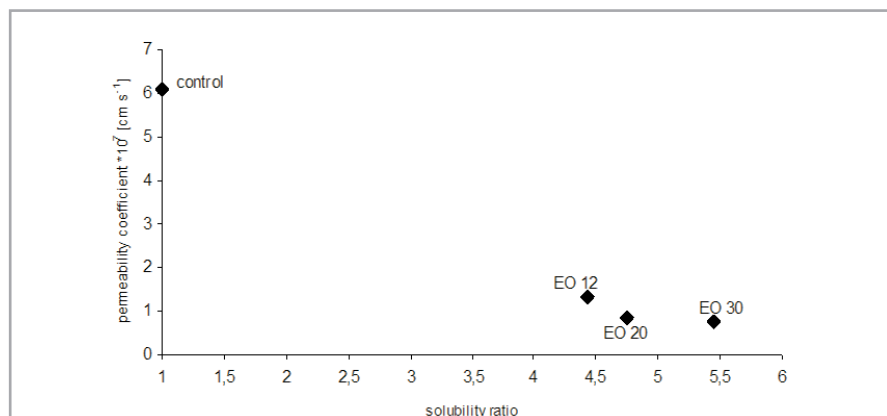The adequate correlation obtained for quercetin displays very similar tendency



**Figure. 7** Permeability coefficients of rutin as a function of solubility ratio in the presence of of polyoxyethylene cetostearyl ethers.
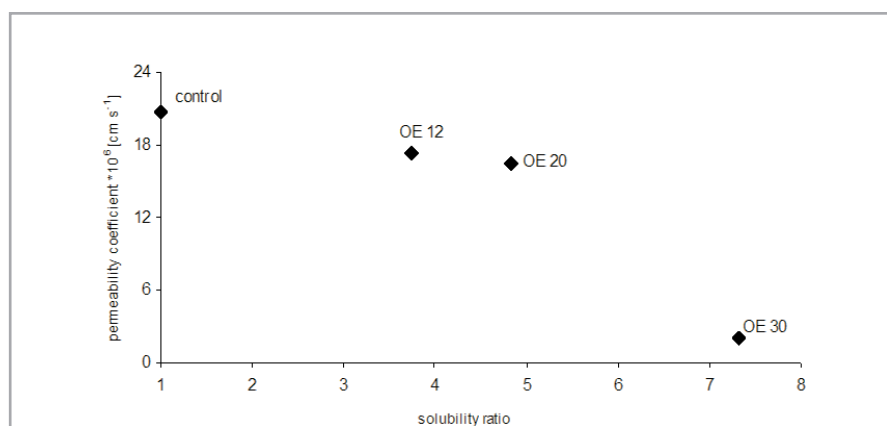


**Figure. 8** Permeability coefficients of quercetin as a function of solubility ratio in the presence of of polyoxyethylene cetostearyl ethers.
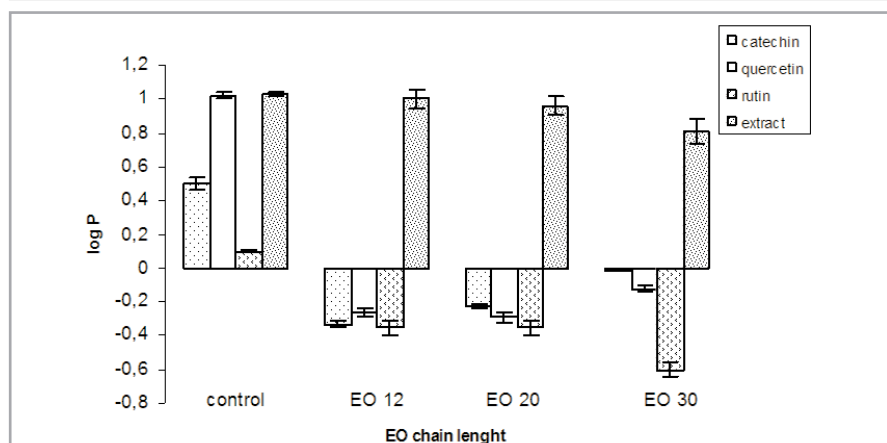


**Figure. 9** Apparent octanol/water partition coefficient of flavonoids in the presence of polyoxyethylene cetostearyl ethers.

- permeability coefficient of flavonoid decreases with increasing value of solubility ratio. However, in the case of quercetin the influence of polyoxyethylene cetostearyl ethers with 12 and 20 EO is not so significant as for rutin.

## Polyoxyethylene alkyl ethers and their effect on apparent

octanol/water partition coefficient of flavonoids.

The other factor that plays a crucial role in the membrane processes is partition coefficient in the octanol / water extraction system. Aiming at elucidation of processes introduced above, we have

determined the effect of polyoxyethy-lene cetostearyl ethers (6%) on apparent octanol/water partition coefficient. The results of this research can be sketched according to the Fig. 9.

The results reported at the Fig.9 allowed as to observe that polyoxyethylene ce-tostearyl ethers display significant influence on the partition coefficient of flavonoids in the octanol / water extraction system. The tendency in similar for all flavonoids – the incorporation of surface active agent to the system causes the decrease in partition coefficient, however, only a little effect can be observed for flavonoids from grape leaf.
The relation between permeation coefficient and octanol/water partition coefficient of flavonoids from grape leaf in the presence of polyoxyethylene cetostearyl ethers has been presented at the Fig. 10.

The highest permeability coefficient can be observed for the control system, which does not contain any surfactant. The ability of flavonoids from grape leaf to penetrate through the lipophilic membrane increases with increasing value of log P. The decrease in the EO chain length results in the increase of both log P and permeability coefficient. The adequate correlation for rutin has been reported at the Fig. 11.

The analysis of Fig. 11 results in a conclusion that has been introduced above – the ability of rutin to overcome the barrier which is lipophilic model membrane is the highest in the system without any surface active agent. The permeability coefficients decrease with decreasing value of log P.

■ Conclusions

The results of the research introduced in this paper have evidenced that non-ionic surfactants are able to change permeation profile of flavonoids.
It has bee documented that presence of polyoxyethylene cetostearyl ethers in the system causes the decrease in permeability coefficient of all investigated polyphenols. The hampering effect of
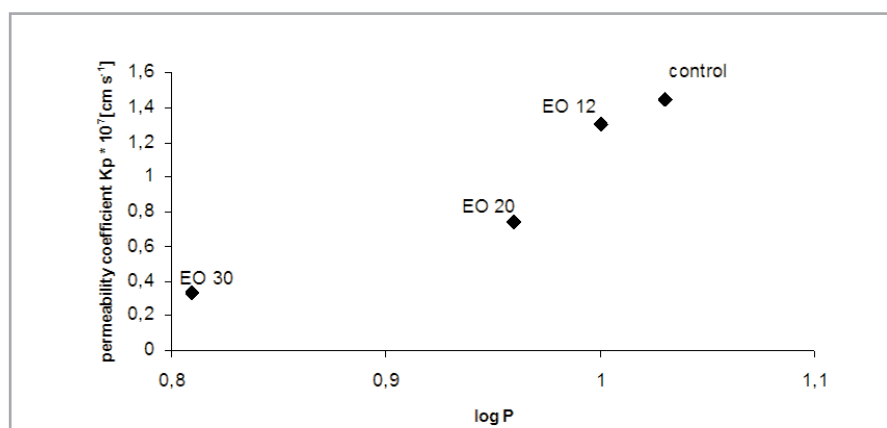


**Fig. 10** Permeability coefficients of flavonoids from grape leaf as a function of log P in the presence of polyoxyethylene cetostearyl ethers.
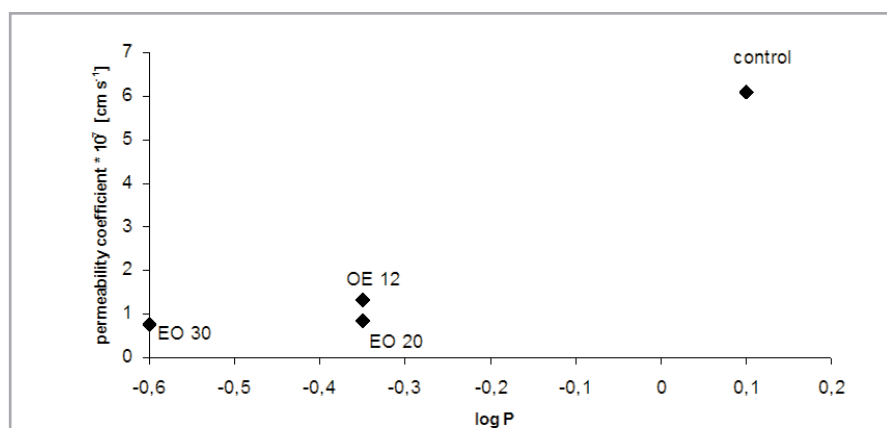


**Fig. 11** Permeability coefficients of rutin as a function of log P in the presence of polyoxyethylene cetostearyl ethers.

surface active agents increases with increasing length of EO chain. Migration rate of flavonoids depends also on concentration of surfactant and decreases with increasing value of concentration. The research carried out on the partition coefficient of flavonoids in octanol/water extraction system have proven that incorporation of polyoxyethylene cetostearyl ethers to the system results in the decrease of partition coefficient. The correlations plotted for rutin and flavonoids from grape leaf has evidenced that the migration rate of flavonoids decrease with decreasing value of log P. The increase in the length of EO chain causes the decrease of both permeability coefficient and log P.

The studies on solubility of flavonoids in the presence of surfactants have shown that all surface active agents incorporated to the system increase the solubility

of flavonoids. The relations determined for rutin and quercetin have proven that permeability coefficient decreases with increasing solubility ratio. Solubility of the permeant in the vehiculum appears as a very important factor determining activity of the compound. The permeation process of the substance is ruled by two main factors: the influence of the surfactant on the membrane permeability and by permeant-solvent affinity. Taking into consideration all results quoted in this paper, we can suppose that changes in the permeant solvent-affinity caused by the surface active agent are the main reason of hampering effect. The addition of surfactant to the system causes the increase in the solubility of the flavonoid molecule what decreases the activity of the substance in vehiculum. Moreover, surface active agents create micelles under these conditions. Thus, the molecule of flavonoid is enclosed in micelle system what results in the de-

crease of its activity and concentration of free molecule in the solution. Mechanisms introduced above can be the reason of the decrease in driving form for the permeation.

The research presented in this paper has proven that surface active agents existing next to flavonoids in the cosmetic formulations should be selected very carefully. Formulators should be aware that these substances are able to influence permeation profile of flavonoids in the market product.

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