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Iontophoretic delivery of marigold extract - formulation studies

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ABSTRACT

Iontophoresis allows the penetration of drugs through the skin by applying a weak electric current called galvanic current, using two poles (one positive and one negative pole). The drug is applied in accordance with its polarity. The use of transdermal administration becomes efficient compared to other routes as it does not decrease drug bioavailability and does not cause stomach irritation, as in the case of oral route and it is not as invasive and painful as the intramuscular and intravenous routes, thus leading to greater patient compliance with therapy. The hydrogels allow the active principle contained in them to undergo the action of ionizing and electrical current, thus being absorbed by the skin. Due to its biocompatible and non-toxic nature, these hydrogels are employed as synthetic dressings used in the treatment of burns and ulcers, as they hydrate and refresh the skin and reduce pain. Rutin (quercetin-3-O-rutinoside) is a flavonoid (polyphenol compounds with a fundamental y-benzopyrene or croman nucleus), traditionally used as an antioxidant and vasoprotective agent, which reduces edema formation and also acts to prevent the damage caused by ultraviolet radiation.
This study analyzed the influence of iontophoresis, irradiation time and the solution concentration on the drug release profile. Hydrogels with different polyvinylpyrrolidone (PVP) concentration and different radiation doses showed the same release profile. Iontophoresis increased the release rate of drugs such as rutin from 100 minutes to 80 minutes.

INTRODUCTION

Transdermal delivery is the most commonly used and most extensively studied route for topical drug delivery [1].

The large surface area of skin and easy accessibility makes it a good site for drug delivery. However, the major hurdle in the development of transdermal delivery devices is to be able to overcome the highly impermeable, keratinized outermost layer of the skin, the stratum corneum. The drugs that most successfully penetrate the skin are those that have a low molecular weight and optimal hydrophilic/lipophilic properties [2].

The introduction of selected functionalities in a polymeric network offers the possibility to modulate the delivery of therapeutics drugs in response to different stimulus such as pH, temperature, electric and magnetic fields. In particular, the use of an electric field has been proposed as a simple method to precisely modulate drug delivery by controlling the applied voltage [3 5], opening novel perspectives for preparing effective transdermal drug delivery.

Hydrogels sensitive to electric currents are usually made with polyelectrolytes, undergoing shrinkage or swelling by turning on an electric field. Sometimes, these hydrogels show swelling on one side and shrinkage on the other side, causing hydrogel bending [6]

Iontophoresis is ideally suited to facilitate the transport of hydrophilic ionizable molecules that are usually not good candidates for passive transdermal delivery expanding the range of drugs that can be considered for administration by the transdermal route, the key advantage of iontophoresis is the control that it affords over delivery kinetics [7]. Under

physiological conditions, the skin acts as a cation-selective membrane (with a pI of ~ 4 - 4.5). Anions are delivered exclusively by electromigration from the cathode [7]. It acts principally on the molecule by introducing a second driving force the electrical potential gradient in addition to the concentration gradient across the skin [8-9]. The rate and extent of drug delivery are determined by the duration, intensity and profile of current application.

The use of transdermal administration becomes efficient when compared to other routes, as it does not decrease drug bioavailability and does not cause stomach irritation, as in the case of oral route and is not as invasive and painful as the intramuscular and intravenous routes, thus leading to greater patient compliance with therapy. However, this technique requires the drug to be water soluble, mostly aqueous, non-precipitating on the skin, without dripping off at the absorption site and allowing ion transference. The ability to modulate delivery via the applied current enables easy individualization of therapy [10-11].

Iontophoresis is particularly suited to peptide and protein delivery, on both physicochemical and pharmacological grounds. They are usually hydrophilic, have good aqueous solubility and contain ionizable groups. All of which are advantageous physicochemical properties from an iontophoretic perspective. They are potent hence, the therapeutic dose is small and their sometimes complex endogenous secretion profiles, e.g., different release patterns during the day or continuous or pulsatile secretion, can be easily mimicked by careful modulation of the iontophoretic current profile so as to simulate normal pharmacological patterns [12 - 13]. More recently, it was shown that transdermal iontophoresis was also able to deliver a functional negatively-charged protein (ribonuclease T1; 11.1 kDa and a pI of 4.27), larger and more complex biomolecules across the skin and provide a more patient-friendly alternative to parenteral administration [14].

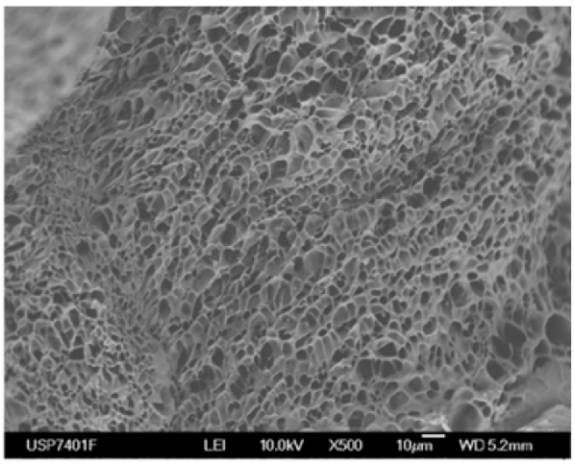

Fig 1. MEV of PVP hydrogels' lyophilized structure

The hydrogels, as PVP hydrogels (Figure 1) adhere to the skin, have a high degree of swelling in aqueous fluids, allowing the active principle contained in them to undergo the action of ionizing and electrical current when using iontophoresis, thus being absorbed by the skin. Due to their being biocompatible and non-toxic, these hydrogels are employed as synthetic dressings used in the treatment of burns and ulcers, because they hydrate and refresh the skin, as well as reduce pain [15].

The therapeutic use of medicinal plants is one of the characteristic features of the human species and it is found in virtually all known civilizations or cultural groups. It is known that all pharmacology is based on active principles of plants. When adequately used, phytotherapic drugs can have therapeutic effects that are more efficient than allopathic medicines, with minimized side effects. Improper use of herbal medicines, such as self-medication, can result in many side effects, such as allergic reactions, toxic effects on various organs and even the development of certain cancers. Herbal medicine, as well as all medications, should offer quality assurance of its therapeutic effects and standardized composition for safety.

Rutin (quercetin-3-O-rutinoside) is traditionally used as an antioxidant and vasoprotective agent, which reduces edema formation and also acts to prevent damage caused by ultraviolet radiation.

Studies have shown that some phytotherapic drugs do not have the tendency to penetrate the epidermis stratum corneum, due to their hydrophobic nature. Recently, the cutaneous permeability of rutin was studied in *in vitro* vertical cell diffusion through skin biomembrane. Rutin carried in the emulsion did not have the tendency to be absorbed during 52 hours of experiment, showing that flavonoid activity is exercised on the superficial layer of the skin, acting as a topical product) [16].

Rutin can become soluble with the introduction of carboxylate or sulfate groups to glycosides' hydroxyl groups, without losing its antioxidant activity. As the iontophoresis technique requires a water-soluble drug, the succinyl was synthetized and used as reference regarding drug release in a study of phytochemicals.

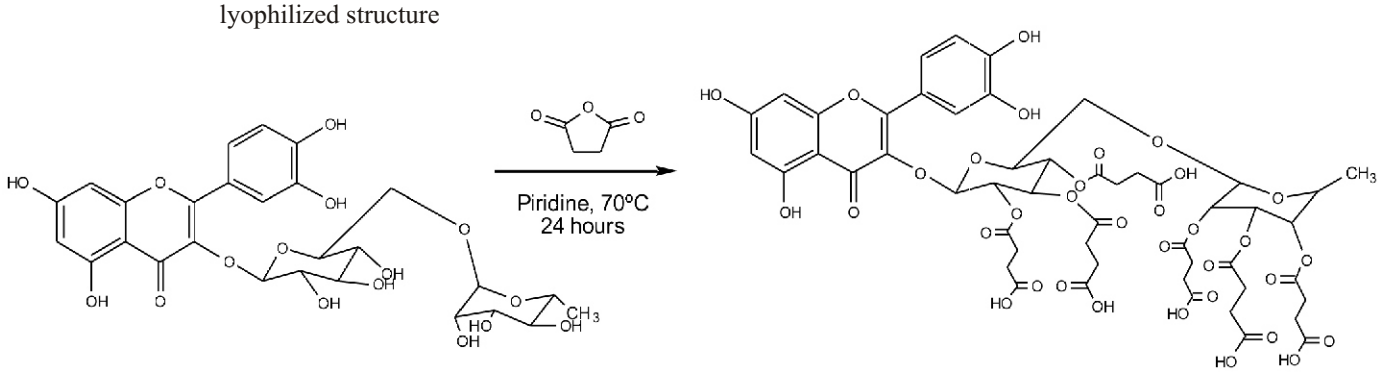

Fig 2. Succinyl rutin synthesis

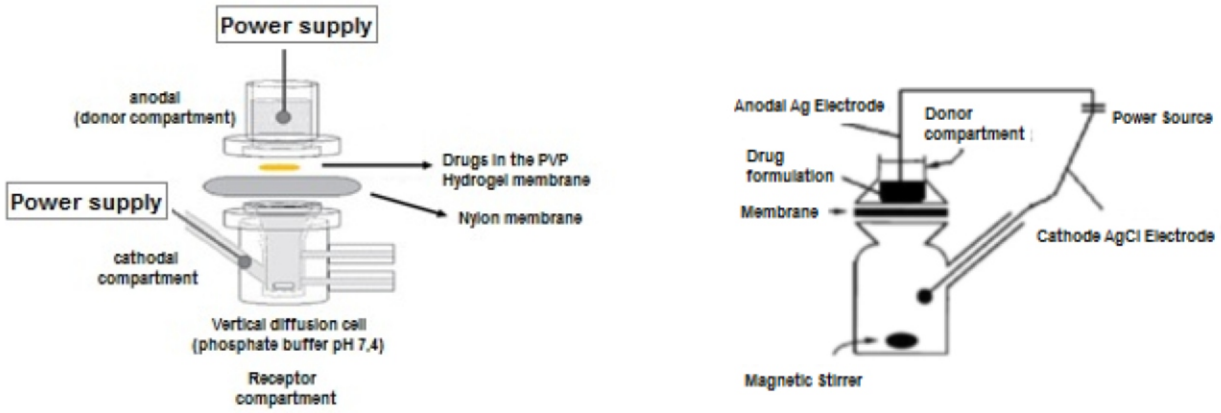

Fig 3. Scheme of iontophoresis apparatus

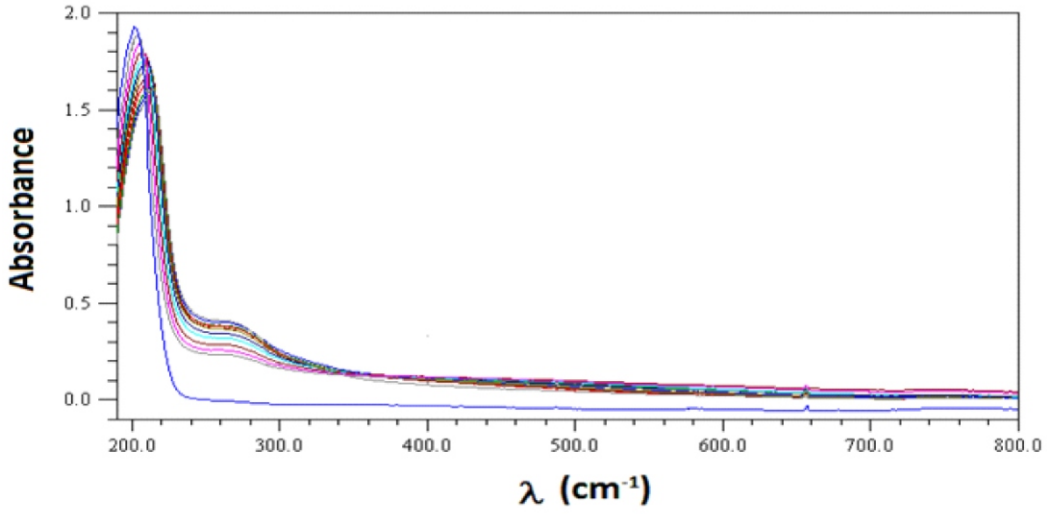

Fig 4. Succinyl rutin release carried out by UV detection

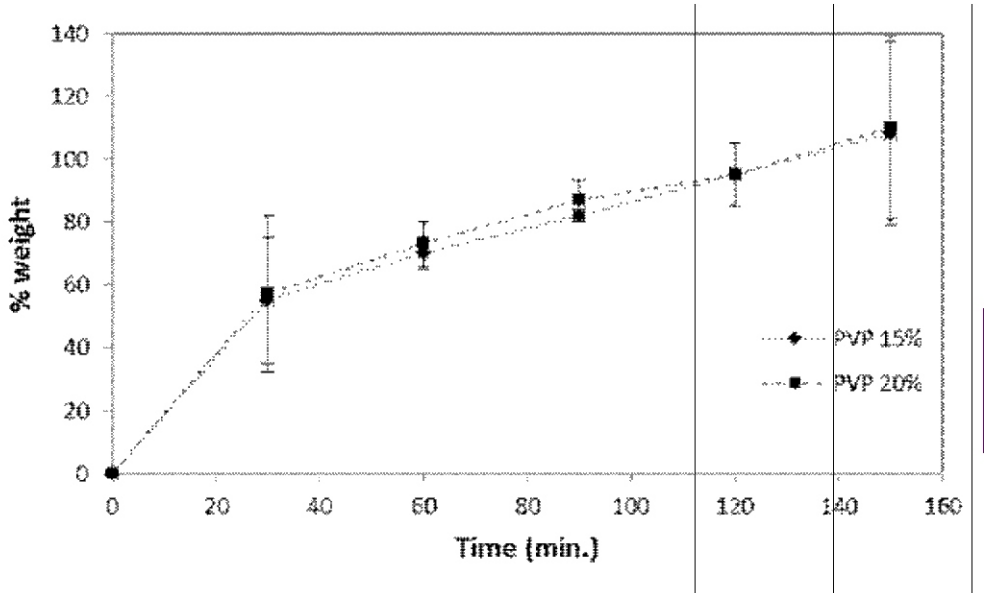

Fig 5. Drug release profile in PVP membranes obtained with different concentrations.

MATERIALS AND METHOD

Chemicals

Rutin (98,8%) was purchased from Natural Pharma (São Paulo, SP, Brazil). Succinic anhydride, pyridine, butanol and ethylic ether were supplied by Synth (São Paulo, Brazil).

Preparation of succinyl rutin

A solution of rutin (5g, 8.2 mmol) and succinic anhydride (7.5 g, 74.94 mmol) in pyridine (200 mL) was heated to 70 °C for 24 hours. After this period, the pyridine was removed by rotoevaporation, then butanol (20 mL) was added and the solution was heated until it was solubilized. After that, the solution was cooled and ethyl ether (20 mL) was added. The solution was vacuum-filtered and washed with 20 mL ethyl ether (twice). After this, it was solubilized in water and lyophilized. Figure 2 shows the synthesis of succinyl rutin.

Preparation of hydrogel membrane with drugs

The hydrogel membrane was prepared from a solution of PVP (10-20%) in distilled water, alcohol or phosphate buffer pH 7.4. The solution was placed into molds and the solvent was evaporated. Thus PVP films are produced. The films were irradiated by UV lamp at 240 nm for two to three hours.

The PVP hydrogel membrane was soaked in 1000µL rutin solution (1 g / mL) for 15 minutes or until full absorption.

Method application: Iontophoresis methodology

Franz cells with nylon membrane were used to simulate drug permeation into the skin. Tests were performed with and without the iontophoresis to verify rutin release profile by iontophoresis (Figure 3).

The samples were analyzed regarding herbal release every 15

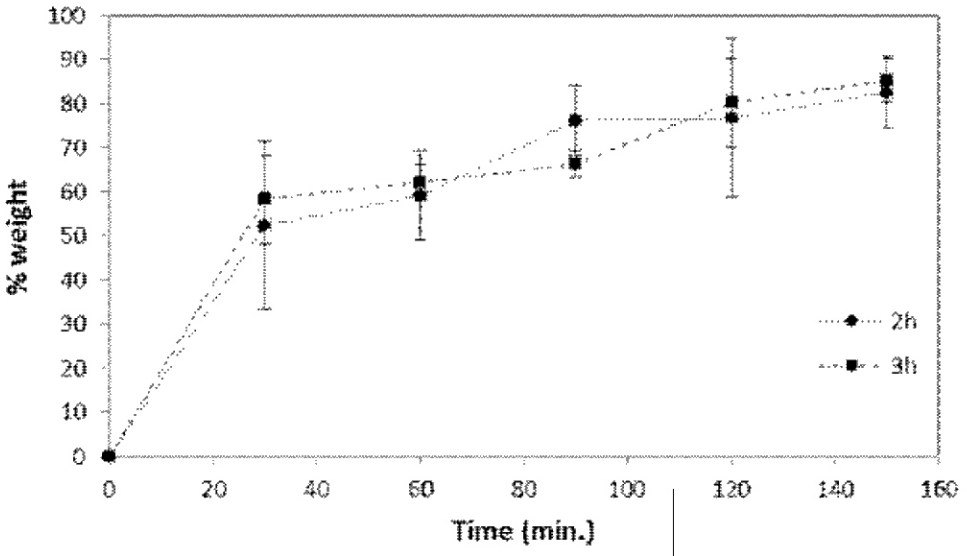

Fig 6. Drug release profile in 100 mg.mL-1 PVP membranes obtained with different irradiation times

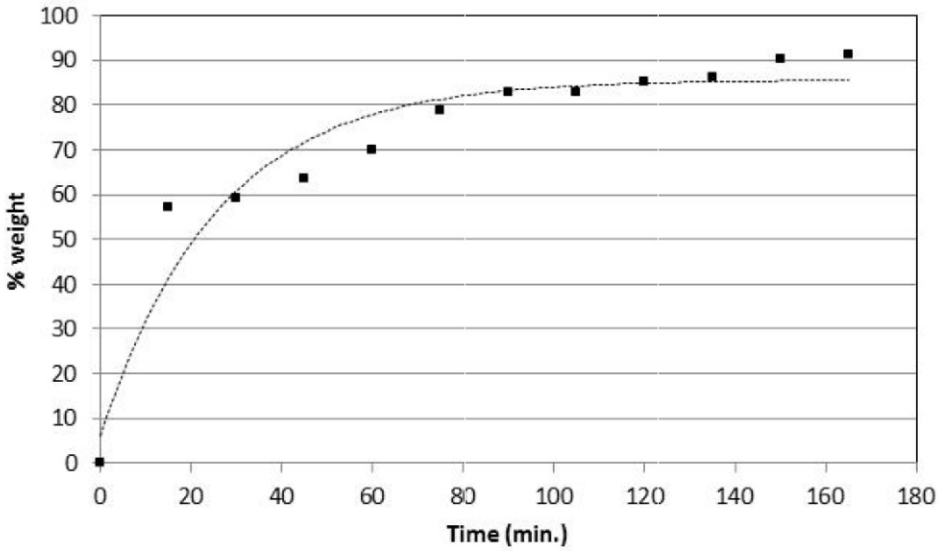

Fig 7. Release curve of succinyl rutin in PVP hydrogel membrane; in vitro assay of release in Franz cells without iontophoresis use.

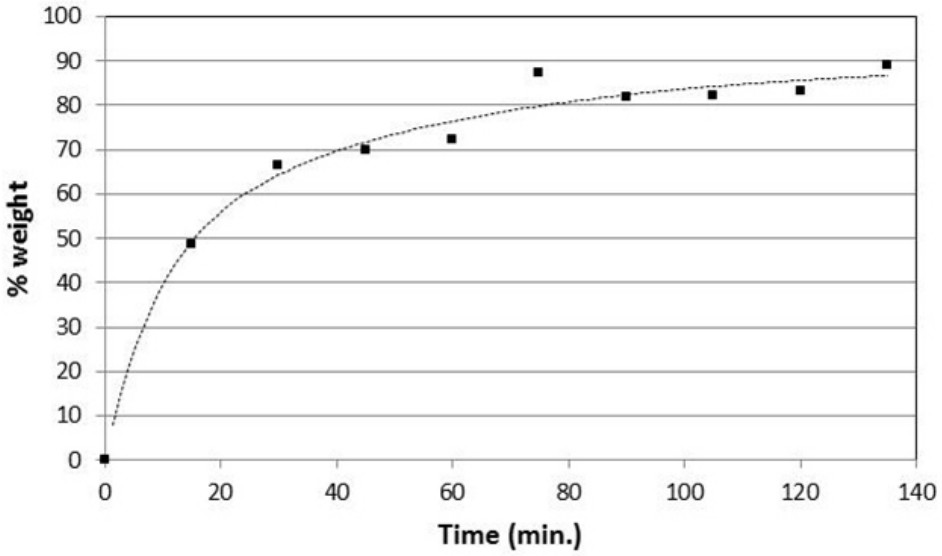

Fig 8. Release curve of succinyl rutin in PVP hydrogel membrane; in vitro assay of release profile in Franz cells with iontophoresis use.

minutes until they achieved a plateau, which indicated that the drug stopped being released. The samples were analyzed by UV absorption spectrophotometer Multi-Spec 1501 to simulate the concentration of drug release based on a standard curve.

RESULTS

The figure 1 shows the PVP hydrogels' lyophilized structure, and can be observed the hydrogel porosity, homogeneity and spongy structure.

The modified rutin release control was performed using UV-Vis spectroscopy. Figure 4 shows the release profile of rutin succinated in a broad range of radiation wavelengths in the ultraviolet in the calibration curve.

Before starting the release studies with and without iontophoresis, parameters related to PVP hydrogel were also analyzed, it was analyzed the release behavior of drug in hydrogel membranes produced by UV radiation by varying the solution concentrations (figure 5) and irradiation time (figure 6). It is observed that the porosity in all cases did not interfere in the release profile. Therefore, the choice of hydrogel work was based on physical stability to adhere the skin.

Two tests were performed with succinyl rutin: release study without and with iontophoresis (Figure 7 and 8 respectively). The iontophoresis would be responsible to assist and increase the drug release. The release profiles observed in both cases are similar; however, in figure 8, with iontophoresis, the drug was released more quickly.

The drug release achieved the plateau in around 80 minutes for the assay with iontophoresis (Figure 8) and 100 minutes for the assay without it (Figure 7). This results shows that succinyl rutin release in PVP membranes is faster with iontophoresis.

DISCUSSION

The UV radiation and irradiation time parameters are important to take into consideration, as they influence in the porosity of the hydrogel, that is responsible for the absorption of aqueous solutions and drug delivery release. The hydrogel porosity and structure are showed in the figure 1

This results from release drug with and without iontophoresis (Figure 7 and 8) shows that succinyl rutin release in PVP membranes is faster with iontophoresis. Similar result was found in studies carried out in Yorkshire swine, investigating the feasibility of delivering zolmitriptan (MW 287.4 Da). These studies show that the drug levels in the blood (7.1 ± 1.7 and 11.9 ± 2.0 ng/mL at t = 30 and t = 40 min, respectively) closely followed the variations in the applied current (4-step profile with current intensities ranging from 0.35 to 0.05 mA/cm2) and extrapolation of the results to humans suggested the feasibility of delivering therapeutically relevant amounts of the drug [17].

Another major double-blind placebo-controlled phase III clinical trial involving a 10-min iontophoretic treatment with either lidocaine or a saline placebo demonstrated that the intensity of pain was lower in adults and children following iontophoresis of lidocaine [9]. Several studies comparing the iontophoretic delivery of fentanyl showed the equivalence of the two methods [18]. In some of these studies, patients reported a preference for the iontophoretic patch because mobility was not restricted [19]. The most common treatment-related were local skin reactions, resolved spontaneously without treatment [20].

The sumatriptan iontophoretic transdermal system was associated with a significantly higher percentage of patients reporting headache pain relief 2 hours post-treatment (52.9% vs. 28.6%, respectively; $P < .0001$) and significantly more patients experienced relief from nausea, photophobia, and phonophobia [21].

Similar results was also found in a study [22] with hybrids gelatin hydrogels tested as electro-responsive delivery systems able to modulate diclofenac sodium salt (DSS) release in response to an applied electric field. This study shows that the drug is dispersed into polymers at a molecular level and higher voltages have resulted in higher current densities than 0.5 mA/cm^2 (human limit), while lower voltages were not able to significantly modify the electrically-induced delivery of DSS by powered samples. They concluded that the increase in the release rate, as well as in the total released drug, is recorded as a consequence of the electrically-induced shrinkage [22].

CONCLUSION

Phytotherapic drugs are generally absorbed by the skin from non-aqueous elements, i.e., in the form of unguents or creams. The modification of non-water soluble rutin into water soluble rutin (succinyl rutin), showed that it is possible to release the drug in an aqueous medium.

Iontophoresis showed great potential to release phytochemicals, provided it is used as a support for drug release through a hydrogel membrane. The hydrogel, besides having the potential to aid in drug release, also helps in the healing process, as it has the same porosity that allows local oxygenation and also has a refreshing effect, because its structure consists of at least 90% water.

Results showed that hydrogels with different PVP concentrations and different radiation doses showed the same release profile and iontophoresis technique helps to increase the release rate of drugs such as rutin.

In sum, data from this study encourage the potential use of iontophoresis technique as a vehicle to introduced hydrophilic drugs easily into the skin. However the results about changes in the drug structure are not conclusive, so it is necessary to complete tests using other techniques such as HPLC, to better understand the behavior of drug release and stability, or electro-responsive microparticles could be inserted into a suitable topical drug delivery device in order to release therapeutics as consequence of an external applied voltage.

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