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# ARCT'ALG ® RELEASE FROM HYDROGEL MEMBRANES

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## ABSTRACT

The hydrogel properties make them attractive for a variety of biomedical and pharmaceutical applications, primarily in drug delivery system. Synthetic hydrogels have been studied to develop new devices for drugs or cosmetic active agents release. Arct'Alg® is an extract derived from red algae biomass which has antioxidant, anti-inflammatory and tissue regeneration stimulant properties. This extract was incorporated to poly(N-vinyl pyrrolidone) (PVP) and poly(vinyl alcohol) (PVA) hydrogel membranes obtained by gamma rays crosslinking technique. The ionizing radiation presents the advantage to occur polymerization and sterilization simultaneously in the same process. The aim of this work was the *in vitro* release kinetic study of Arct'Alg® from hydrogel membranes during 24 hours to verify the possibility of use in cosmetic and dermatological treatments. Results showed that about 50% and 30% of incorporated Arct'Alg® was released from PVP and PVA hydrogel membrane devices respectively.

## 1. INTRODUCTION

Controlled drug-delivery systems are designed to deliver the drugs at desirable times and/or specific sites to achieve the therapeutic objective and hydrogels are one of the upcoming classes of polymer-based controlled release drug delivery systems [1-2].

Polymeric hydrogels are hydrophilic polymer networks that may retain a large amount of water and exhibit a semi-solid morphology. The hydrophilic three-dimension network formed by chemical or physical crosslinking can be considered as an ideal candidate for the controlled drug release matrix [3].

Hydrogels can be obtained by gamma radiation of PVP or PVA water solutions. Use of radiation for the formation and modification of hydrogels for biomedical purposes has some general advantages as sterilization of products and a pure product non-contaminated with ballast materials or residuals of toxic initiators [4].

The physical properties of hydrogels make them attractive for a variety of biomedical and pharmaceutical applications [5]. They have been used by many investigators in controlled-release drug delivery systems because of their good tissue compatibility and easy manipulation of swelling level and, thereby, solute permeability [6].

In this work, the active principle Arct'Alg® was incorporated to PVP and PVA hydrogel membranes. The aim was the *in vitro* release kinetic study of Arct'Alg® from hydrogel membranes during 24 hours to obtain a device for the treatment of tissue regeneration processes.

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Raw materials used for synthesis of polymeric matrices

Poly (N-vinyl-pyrrolidone) (PVP) K 90, Kollidon® 90F, average molar weight from 1000000 to 1500000 from BASF, poly (ethylene glycol) (PEG 300) from Oxiteno, agar technical type 3 from Oxoid and poly (vinyl alcohol) (PVA), Celvol® E47/88, degree of hydrolysis 87-89%, melting point 180°C, glass transition temperature 58ºC, from Dermet Agekem.

#### 2.1.2 Active principle

Arct'Alg® is an yellow extract from red algae provided by Exymol. It has about 7.5% of dry weight, composed by about 7% of dipeptide citrullyl -arginine, 0.15% of taurine amino acid, 0.14% of methyl parabens and minerals.

### 2.2 Methods

#### 2.2.1 Preparation of hydrogel device

Two formulations were used to obtain the hydrogel devices. The components and their concentrations are described in the Table 1.

**Table 1. Description of the formulation of PVA and PVP hydrogel devices.**

| MATRIX | COMPONENTS | CONCENTRATION (%) |
|---|---|---|
| | PVP K90 | 6.0 |
| **PVP** | PEG 300 | 1.5 |
| | ÁGAR | 0.5 |
| | ARCT'ALG® | 3.0 |
| **PVA** | PVA | 8 |
| | ÁGAR | 1 |
| | ARCT'ALG® | 3 |

First, the solutions of PVP and PVA hydrogel were prepared. For PVP hydrogel were mixed PVP K90, PEG 300 and water. After 24 hours at room temperature, this solution was heated

and added to the agar keeping in heat until complete dissolution of the components. The hydrogel of PVA was synthesized from the mixture of PVA and water. The PVA water solution was heated for complete dissolution and the agar was added under heat until dissolution of the components.

After cooling the hydrogel solutions at approximately 40ºC, 3% of Arct'Alg® was incorporated in each of the formulations and mixed until complete homogenization.

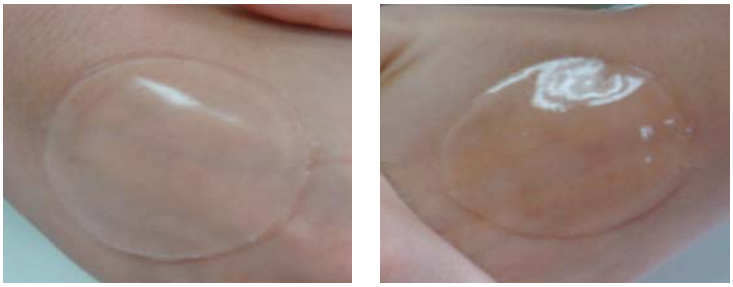
The membranes devices were prepared pouring 5mL of the hydrogel solution containing the active principle in circular packs, which were sealed, packed and then irradiated with gamma rays from a source of $^{60}Co$, dose rate of 5,72 $kGY/h^{-1}$. The PVP devices were irradiated at a dose of 25 kGy and the PVA at a dose of 20 kGy.

### 2.2.2 *In vitro* release kinetics and quantification of Arct'Alg®

PVP and PVA hydrogel membranes devices were cut in half and immersed in 35mL of PBS 0.1 M pH 5.0 in glass flasks, in triplicate, and placed in an incubator under constant agitation at 37°C. Aliquots of 1mL were collected every hour during the first 7 hours of the test, and then, after 24 hours. The quantification of released Arct'Alg® were determined by HPLC equipment from Shimadzu, equipped with a binary pump and controlled with the CLASS-LC10 software. A total of 100 µL of samples were injected into a reversed-phase C18 column (250 mm x 4.6 mm) from Varian, the flow rate was 1 mL/min and the UV detection was performed at 200 nm. The mobile phase consisted of 3% acetonitrile and PIC B6 97% from Waters in an isocratic system. The released amount of Arct'Alg® was calculated by comparing the peak area of citrullyl-arginine present in Arct'Alg® released with the standard peak area obtained by injection of 5 µL of citrullyl-arginine (0.2 mg/mL) standard solution.

## 3. RESULTS AND DISCUSSION

The obtained PVA and PVP hydrogel devices as membranes were evaluated visually and by touch. The criterion for considering the membrane devices appropriate or not to be subjected to the release kinetics was the formation of a homogeneous membrane, transparent, flexible and soft, with mechanical properties suitable for handling as shown in Fig. 1.



PVA device PVP device

**Figure 1. PVA and PVP hydrogel membrane devices**

The *in vitro* release kinetic was performed to determine the Arct'Alg® release profile from the obtained PVP and PVA devices.

To verify the release of Arct'Alg®, extract composed of various substances, was used the dipeptide citrullyl-arginine as standard, one of its main constituent. The Fig. 2 presents the standard solution chromatogram where shows the citrullyl-arginine peak in the retention time of about 8 min.

The quantification of released citrullyl-arginine was obtained by comparing the peak area with that showed by the standard solution and the release kinetic results are presented in Table 2.

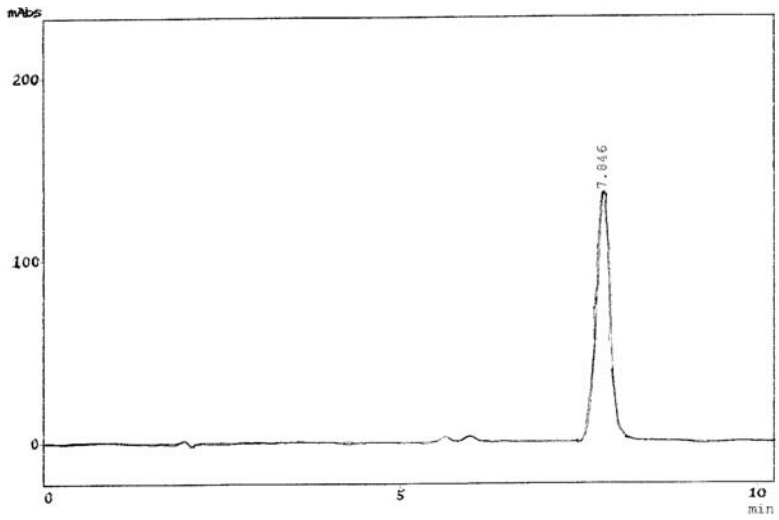



**Figure 2. Chromatogram of citrullyl-arginine standard solution**

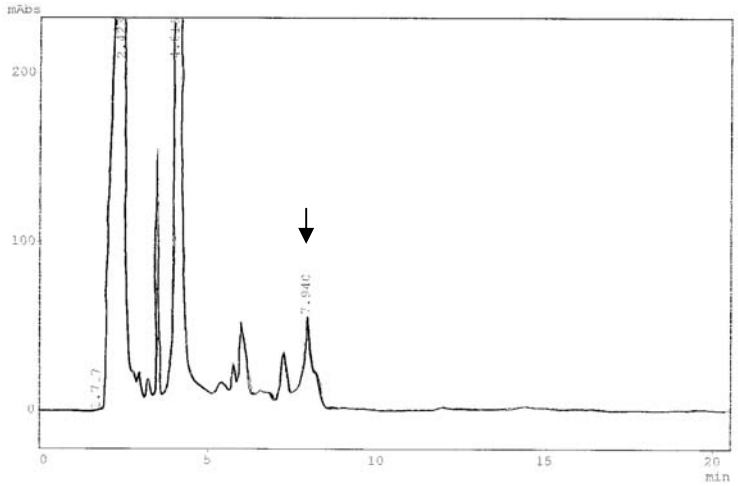



**Figure 3. Chromatogram of Arct'Alg® release kinetics by PVP device after 1 hour of release.**

The chromatograms obtained after 1 hour of release by PVP and PVA hydrogel devices are shown in Fig. 3 and 4 respectively. In these figures it can be seen a peak in the retention time of about 8 min, corresponding to citrullyl-arginine.

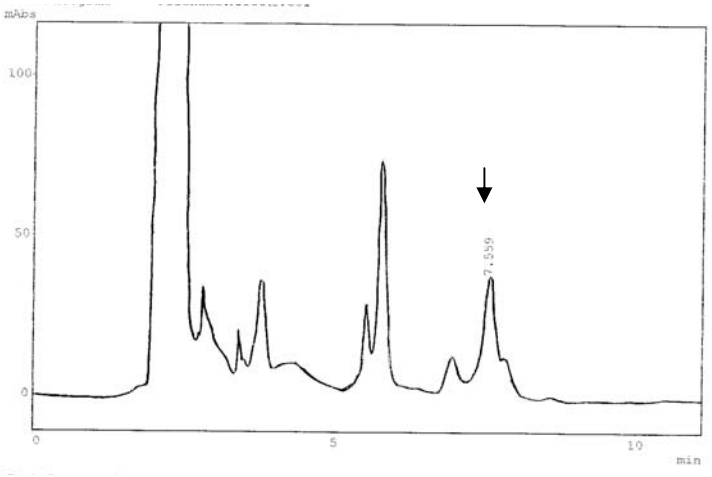



**Figure 4. Chromatogram of Arct'Alg® release kinetics by PVA device after 1 hour of release.**

**Table 2. Release kinetic results of PVP and PVA hydrogel devices, containing Arct'Alg® analyzed by HPLC.**

| Device | Release time | Retention time | Peak area | Citrullyl-arginine |
|---|---|---|---|---|
| | (h) | (min) | | (µg/mL) |
| | 1 | 7.94 | 1102231 | 6.009 |
| | 2 | 7.48 | 938004 | 5.114 |
| | 3 | 8.02 | 916417 | 4.996 |
| **PVP** | 4 | 7.59 | 824082 | 4.493 |
| | 5 | 7.96 | 839009 | 4.574 |
| | 6 | 7.56 | 828229 | 4.515 |
| | 7 | 7.51 | 818832 | 4.464 |
| | 24 | 7.60 | 842494 | 4.593 |
| | 1 | 7.55 | 667615 | 3.639 |
| | 2 | 7.66 | 653028 | 3.560 |
| | 3 | 7.59 | 626414 | 3.415 |
| **PVA** | 4 | 7.64 | 621342 | 3.387 |
| | 5 | 7.59 | 616679 | 3.362 |
| | 6 | 7.61 | 503148 | 2.743 |
| | 7 | 7.61 | 509028 | 2.775 |
| | 24 | 7.59 | 493516 | 2.690 |

The release profile of the PVP and PVA devices are shown in Fig. 5 and 6. The devices release profile shows that the maximum citrullyl-arginine release was in the first hour, with no increase in the release level up to 24 hours. These results suggest that Arct'Alg® contained in the PVA and PVP membranes was completely released just in one hour.

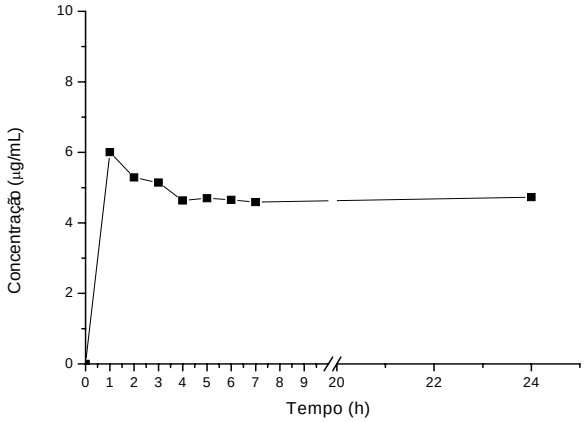



**Figure 5. Release profile of citrullyl-arginine contained in Arct'Alg® released by PVP hydrogel device.**

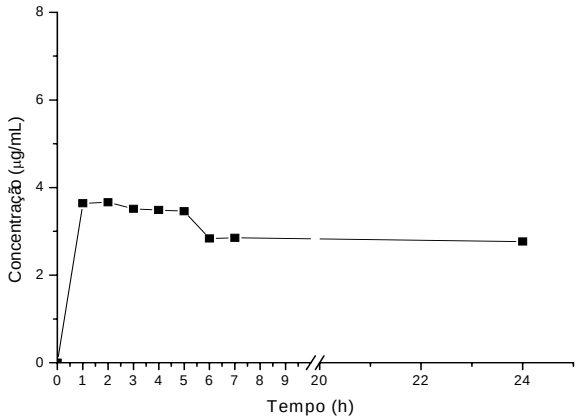



**Figure 6. Release profile of citrullyl-arginine contained in Arct'Alg® released by PVA hydrogel device.**

As known, in the composition of Arct'Alg® has 7.5% of dry residue which 7% of this is citrullyl-arginine. Based in this information it could be calculated the released percentage of immobilized Arct'Alg®. The PVP hydrogel device has released about 50% of total immobilized and the PVA device about 30% as can be seen in Table 3. Probably this released

amount was the total active principle in the hydrogel membrane device. It could be due to irradiation process which could therefore cause damage to Arct'Alg® components, reducing in 50 and 70% the citrullyl-arginine amount in the final hydrogel membrane devices.

**Table 3. Arct'Alg® released from hydrogel devices**

| Hydrogel Device | Release Time (h) | Citrullyl-Arginine (mg) | Released ARCT'ALG® (%) |
|---|---|---|---|
| | 1 | 0.421 | 53.3 |
| | 2 | 0.370 | 46.8 |
| | 3 | 0.359 | 45.6 |
| PVP | 4 | 0.324 | 41.1 |
| | 5 | 0.329 | 41.6 |
| | 6 | 0.325 | 41.1 |
| | 7 | 0.321 | 40.7 |
| | 24 | 0.330 | 41.6 |
| | 1 | 0.255 | 32.2 |
| | 2 | 0.256 | 32.4 |
| | 3 | 0.246 | 31.2 |
| PVA | 4 | 0.244 | 30.9 |
| | 5 | 0.242 | 30.6 |
| | 6 | 0.198 | 25.2 |
| | 7 | 0.199 | 25.3 |
| | 24 | 0.194 | 24.5 |

## 4. CONCLUSION

The study of the release kinetic of Arct'Alg® showed that the incorporation of this active principle in hydrogel one step formulations is possible in spite of release reduction in about 50% or 70%.

The PVP hydrogel membrane device showed a greater release of Arct'Alg®, around 50%, compared with 30% PVA hydrogel membrane device. Therefore, through the results, it can be concluded that the PVP device probably is the most suitable for dermatological and/or cosmetic uses due to antioxidant, anti-inflammatory and tissue regeneration stimulant Arct'Alg® properties.

Studies must be continued for Arct'Alg® immobilization in hydrogel membrane device by hydrogel swelling property to verify if there is an increase in the release profile of active principle.

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