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HYSTERESIS OF CONTACT ANGLE. DYNAMIC WETTABILITY STUDIES OF COLLAGEN AND DOXYCYCLINE POROUS MATRICES CROSSLINKED WITH TANNIC ACID

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Collagen porous matrices are promising delivery systems which offer the possibility to obtain a local optimized drug release. One important prerequisite in understanding the drug dissolution profile is an adequate monitoring of the porous collagen matrices surface properties and surface wettability degree. In this study we have considered direct measurements of the contact angle and hysteresis of contact angle (dynamic contact angle) for some collagen matrices with doxycycline, cross-linked with tannic acid, in order to better describe the wettability properties of these drug release systems. The matrices were obtained by freeze-drying of collagen gels (the release support) which have embedded doxycycline as model drug. These systems were prepared at pH=3.8, and crosslinked with different concentrations of tannic acid (4%, 5%, 10%, respectively 20%). We also took into account in the study an uncrosslinked matrix (without tannic acid) as control sample. A KSV Instrument CAM 101 equipped with a digital camera and the pendant drop method were used for contact angle and surface properties experiments. The liquid (water) is imbibed into the porous matrices producing the contact angle decrease in time. The Young-Laplace equation was applied and the contact angle hysteresis was evaluated (difference between the maximum and the minimum contact angle values) to characterize the surface wettability and hydrophobicity.

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1. Introduction

The contact angle is considered as an useful indicator for a solid surface characterization, providing valuable information on wetting properties, hydrophobicity measure of interfacial properties or adsorbtion phenomenon. Generally, wetting involves the interaction of a liquid with a solid and includes the formation of a contact angle [1] at the solid/liquid/fluid interface, the spreading of liquid over the surface, or penetration of a liquid into a porous medium [2]. For topical systems as collagen porous matrices loaded with different drugs, the contact angle and the hysteresis of the contact angle play important roles in systems biocompatibility, on one hand, and in drug local controlled release, one the other hand [3].

It is well known that collagen represents one of the most favourable matrix for on-site drug delivery, due to its excellent biocompatibility, well established safety profile, high biodegradability and very week antigenicity [4-6]. Collagen sponges (also called porous matrices)

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are used in burns, wounds and plastic surgery [7,8], as well as in parodontology [9]. Collagen - gel formulations with liposomes are applied as controlled transdermal drug delivery systems [10]. Collagen enormous therapeutic potential is completed today with protein/gene delivery systems and tissue engineering [11,12].

The aim of this paper was to evaluate the contact angle and contact angle hysteresis for a complete wettability characterization of porous collagen matrices. These matrices consist in type I collagen loaded with doxycycline hyclate – an antibiotic used for local treatment of periodontal disease and crosslinked with a natural agent, tannic acid [13]. Tannic acid is also used for its synergic antibacterial and astringent activities [14,15]. These collagen porous matrices were designed for on-site treatment of periodontitis disease, as well as for other skin injuries (burns, wounds) and were previously described and characterized [16-18]. The wettability studies come to complete the characterization of these innovative drug release systems.

2. Theoretical background

Contact angle (θ) is a quantitative measure of the solid wetting by a liquid. Geometrically it is defined as the angle formed by a liquid at the three-phase boundary where a liquid, gas and solid intersect (Figure 1).

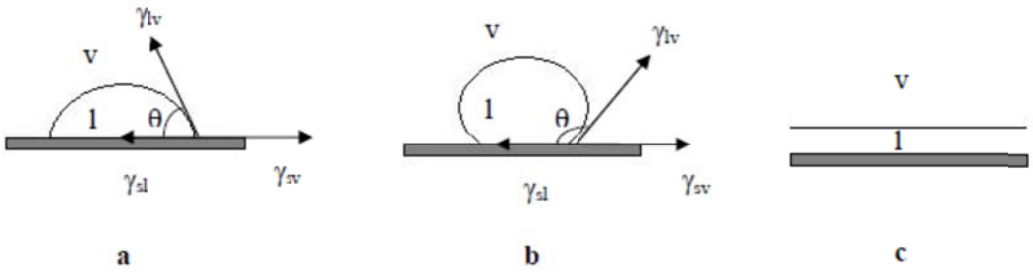


Fig. 1 - Contact angle between a liquid (l) and a flat solid surface (s): (a) and (b) corrrespond to partial wetting ($\theta > 0$) and (c) corresponds to complete wetting ($\theta = 0$).

For a small liquid drop deposited on a solid surface, three interfacial tensions are involved γ_{sv}, γ_{sl}, γ_{lv}, the solid-vapour, solid-liquid and liquid-vapor respectively. The force balance giving the equilibrium contact angle is defined by Young's well known relation:

$$\gamma_{lv}.\cos\theta = \gamma_{sv} - \gamma_{sl} \qquad \text{(eq. 1)}$$

In the case of imperfect, non-homogenous solid surfaces, and for porous matrices, a range of contact angles is usually obtained, thus the term *apparent contact angle* is preferred. The maximum value for contact angle, θ_a is called *advancing* and the minimum value, θ_r is called *receding*. The difference between advancing and receding contact angles is called *hysteresis* and is directly related to the extent of surface heterogeneity, pore size, porosity and interconnectivity for macroporous matrices or sponge [1, 19,20]. All these properties are directly related to water adsorbtion and drug release rate from the porous matrices loaded with drug and designed for topical administration [16,17].

3. Materials and methods

Manufacturing process for porous collagen matrices with doxycicline and crosslinked with tannic acid. The process was previously reported [13,21,22]. Briefly, the collagen hydrogels based on 1.2% collagen and 0.2% doxycycline hyclate (reported to the collagen gel) were obtained at pH=3.8. The hydrogels were crosslinked with different amounts of tannic acid (0, 4, 5, 10 and 20% reported to the dry collagen). A program of freeze-drying

(lyophilization), using the Delta 2-24 LSC Christ lyophilizer (Germany) was applied to obtain the porous collagen matrices, codified as shown in **Table 1**.

Table 1Codification of the porous collagen porous matrices.

Collagen porous matrix	Tannic acid %	Doxycycline %	pH
CDA	0%	0.2%	3.8
CDT4	4%	0.2%	3.8
CDT5	5%	0.2%	3.8
CDT10	10%	0.2%	3.8
CDT20	20%	0.2%	3.8
CT4	4%	0%	3.8
CT5	5%	0%	3.8
CT10	10%	0%	3.8
CT20	20%	0%	3.8

Determination of dynamic surface wettability. The contact angle was determined and analysed at room temperature, with a KSV Cam 101 Scientific Instrument, equipped with a digital video camera for images capturing (Helsinki, Finland) [23,24]. The pendant drop dynamic method was applied, using distilled water. The drop shape was monitored with the digital camera, for a time interval up to 12s, and the contact angle, drop diameter, drop height and volume were recorded. The drop shape was mathematically described by the Young-Laplace equation (eq.1) and the contact angle was determined as the slope of the contour line at the three-phase contact point. The dynamic drop method provided advancing and receding contact angles as function of time. At least six independent measurents on different sponge surface locations (both sides) were averaged.

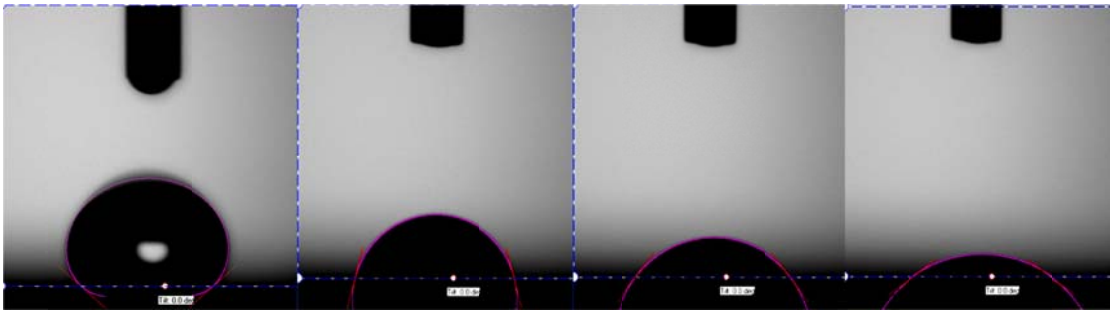
4. Results and discussions

Collagen porous matrices were evaluated for their surface wettability (contact angle measurements) and contact angle hysteresis was determined. As summarized in Table 2, the values for all contact angle were less than 90°, indicating a good hydrophilicity and wettability degree for the top surface.

Table 2. Results of contact angle experiments for collagen porous matrices

Collagen porous matrix	Contact angle θ (˚)	Hysteresis Δθ (˚)
CDA	58.72±1.25	84.72±1.28
CDT4	62.13±1.06	31.77±1.22
CDT5	64.96±1.19	23.55±1.16
CDT10	84.37±1.39	7.23±1.12
CDT20	73.55±1.03	10.86±1.09
CT4	57.07±1.11	35.59±1.23
CT5	60.03±1.24	26.93±1.13
CT10	72.43±1.15	12.66±1.01
CT20	67.28±1.22	15.26±1.03

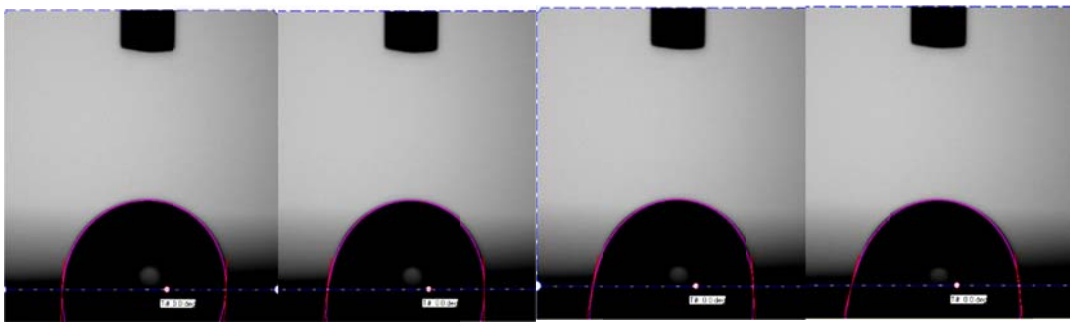
In the Figs. 2 and 3 images of the drop shape for the decrease in time of the contact angle, or hysteresis from image (a) to (d), are exemplified for two types of porous matrices: CDA and CDT10.



a) at 0.00s b) at 1.82s c) at 5.47s d) at 9.72s

Fig. 2. Images for drop shape, contour line for contact angle calculation, hysteresis of contact angle at different time frame, for the collagen porous matrix CDA (with Doxycycline and without tannic acid)

As we expected, the highest value of contact angle hysteresis was observed at CDA-collagen porous matrix without crosslinking agent (almost 85° in approximately 10s). Actually, the drop was infiltrated into the porous medium in 10s (Fig 2).



a) at 0.00s b) at 1.82s c) at 5.47s d) at 9.72s

Fig. 3 – Images for drop shape, contour line for contact angle calculation, hysteresis of contact angle at different time frame, for the collagen porous matrix CDT10 (with Doxycycline and crosslinked with tannic acid 10%).

For CDT10 collagen matrices, the highest value for contact angle was observed, indicating a low hydrophylicity for the porous matrix surface and a small wettability degree. The contact angle hysteresis was also small, while both the drop contour and drop volume were almost constant in time (Fig. 3).

Increasing the crosslinking agent concentration (tannic acid) from 4% to 20%, an increase of contact angle (hydrophylicity decrease) and a contact angle hysteresis decrease occurred, for the collagen porous matrices with and without doxycycline (Figure 4 and 5).

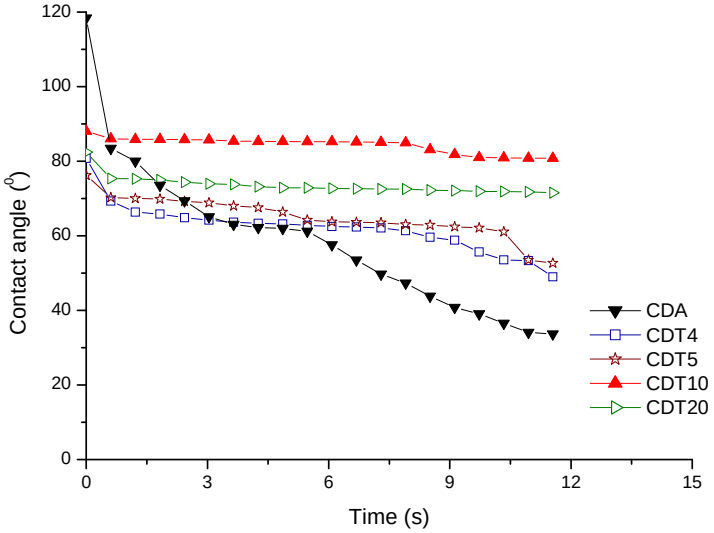


Fig. 4 – Variation of contact angle for collagen porous matrices without tannic acid (CDA) and for cross-linked matrices with different concentration of tannic acid (4, 5, 10 and 20%).

The exceptions for this linear correlation were noticed for the collagen porous matrices with tannic acid 10% (CDT10 and CT10). For these matrices higher values for contact angle (84.37° and 72.43°, respectively) and lower hysteresis (7.23° and 12.66°, respectively) were recorded compared to those obtained at 20% concentration of tannic acid.

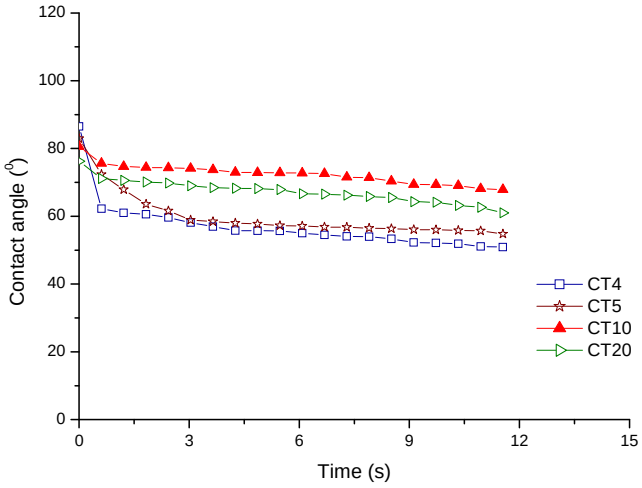


Fig. 5 – Variation of contact angle for collagen porous matrices without doxycycline, cross-linked with different concentration of tannic acid (4, 5, 10 and 20%).

These experimental data correlate very well with our previous studies regarding the viscosity of corresponding hydrogel formulations of collagen with doxycycline and tannic acid [16]; the concentration of 10% of tannic acid induced the highest level of cross-linking, the pore size, pore distribution and surface characteristics being directly related to the concentration of tannic acid as cross-linking agent. As in the scanning electron microscopy previous studies, the most uniform structure for porous matrices was obtained for a level of 10% of tannic acid [16], but the hidrophylicity and wettability degree were at lower levels.

For the porous matrices without doxycycline, no evident decrease in hysteresis of contact angle was noticed (for example, from 35.59° at CT4 to 12.66° at CT10). Although doxycicline had its own effect of cross-linking agent [16,17] upon the surface hydrophilicity and wettability degree, the effect of tannic acid is prevalent.

In Fig. 6 the variations in contact angle and contact angle hysteresis for the collagen porous matrices investigated in this study are synthetically illustrated.

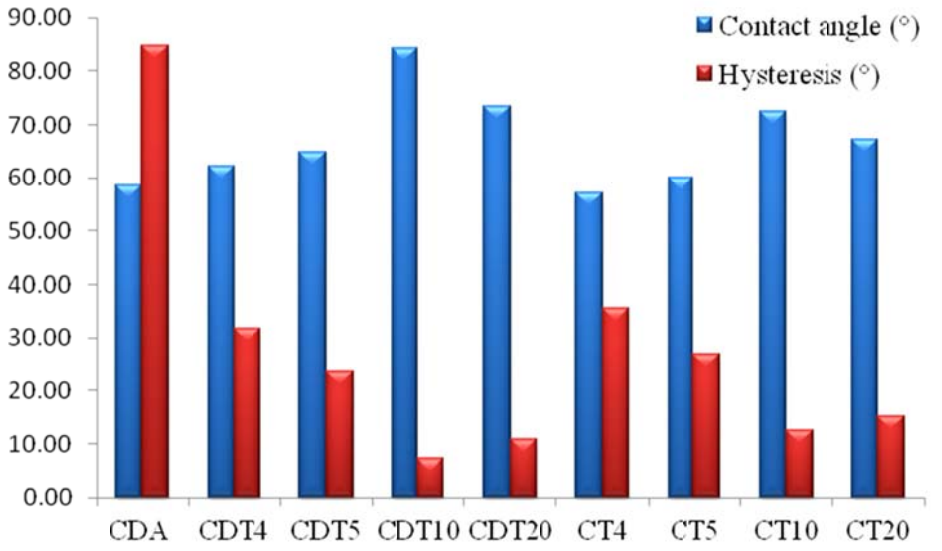


Fig. 6. Contact angle and hysteresis for the collagen porous matrices as indicator of wettability and hydrophilicity of the surfaces

As it can be seen all the porous matrices have proved a satisfactory hydrophilicity (around 67°). The hysteresis of contact angle shows a large interval of variation between 84.72° and 7.23°. The smallest values for hysteresis were recorded for CDT10 and CT10, respectively; for this concentration tannic agent induces the highest crosslinking level for collagen. Many of the porous matrices presented average values for hysteresis around 22□ that correspond to a good wettability degree.

5. Conclusions

Porous collagen matrices containing doxycycline crosslinked with tannic acid were investigated from membrane surface hydrophilicity and wettability degree point of view. Higher hydrophilicity can accelerate the water permeation and improve drug diffusion rate throught the porous matrices.

The contact angle experiments and recorded contact angle hysteresis have indicated that the crosslinking agent concentration as well as the matrix porosity are the major factors that influence the surfaces properties. The matrices containing 10% tannic acid (crosslinking agent) have shown the lowest hydrophilicity and wettability degree. This concentration of crosslinking agent could be considered as a starting point for the optimization of these promising drug systems.

Some models could be further established and further investigations could be developed to correlate the dynamic wettability studies with porous matrices formulations, and drug release studies.

Acknowledgements

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