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The use of differential scanning calorimetry to study the effects of gentamycin on fibrous collageneous membranes

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Abstract

The protein collagen is the most predominant and important protein of the skin and therefore its physicochemical and thermal properties are important to be known. DSC has been applied in order to study the thermal changes caused by using different concentrations of the gentamycin antibiotic in fibrous membranes FM, named AMATCOL at different scanning rates. The thermal effect consisting of several peaks of the fibrous collageneous membrane alone or with different percentages of drug is simplified to a broad peak after 24 h equilibration time. The 35–70 °C endothermic effect attributed to the collapse of the tripple-helical domain of collagen due to the dehydration is affected by the presence of gentamycin. The endothermic peaks due to vaporization of bound water at the temperatures of 90–120 °C are also affected by the presence of gentamycin. This region consists of two peaks at low percentage of gentamycin and at higher percentages the peak near 120 °C decreases in intensity and finally disappears. The re-absorption of the water is more significant in the preparations containing gentamycin after 24 h equilibration time indicating that antibiotic makes a more stable complex with collagen molecules aiding this process. A minimum of re-absorption occurs when the concentration of gentamycin is 2% w/w in accordance with pore size and nitrogen gas permeability measurements. The collagen denaturation occurs at higher temperature when gentamycin is incorporated in fibrous membranes FM. This is an evidence that gentamycin stabilizes the cross-linkings between structural units (covalent, hydrophobic links) due to its interactions with collagen and water. Data resulted from differential scanning calorimetry (DSC) have been corroborated with those resulted from the porosity analysis. Specific morphology of fibrous collageneous membranes FM structure, containing macro-, micro-, and nano-pores resulted from freeze drying, acts on the gas and water vapor permeability, as well as water absorption. These characteristics are important in trans-dermal carriage of gentamycin contained in FM membranes.

Keywords: Collagen type 1; Fibrous membranes; Gentamycin; Thermo-oxidation

1. Introduction

Differential scanning calorimetry (DSC) is a thermodynamic technique suitable for studying phase transitions of various materials. Diagnostic parameters used in DSC are Tm, $T\mathrm{m}_{1/2}$ and ΔH. Tm shows the maximum of the phase transition, $T\mathrm{m}_{1/2}$ the half-width in the middle of the phase transition, and ΔH is the enthalpy of the transition measured as the area under the peak. DSC has been used extensively to follow the phase transitions of biologically important systems such as lipid bilayers and to obtain information about the interactions of drugs on model membranes [1–3]. DSC is a suited technique to study physicochemical properties of collagen, such as the preservation of its triple-helical structure and detect of its denaturation [4]. Numerous studies have been performed aiming to check if fibrous materials are structurally preserved after a certain treatment. As an

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example, DSC is used in the characterization of collagen in acellular bovine cardiovascular tissues, fresh or glutaraldehyde, treated, and stored in different solutions. Samouillan et al. found that the glutaraldehyde treatment followed by octanol storage preserves the tripple-helical conformation of the polypeptide chains of collagen, contrary to the ethanol and PBS storage that induce drastic changes in the thermal and dielectric behaviour of the protein. Moreover, this particular treatment stabilizes the collagen structure by shifting towards high temperature of the collagen denaturation and stiffening of the chains by a cross-linking action [5–7]. DSC was used to study the thermal properties with drug delivery systems containing collagen [8–12]. Collagen-based materials have scaffold properties to support bioactive molecules such as growth factor [13–14]. Insoluble collagen has been utilized as a base material for parenteral drug carrier system and DSC was utilized in order to detect collagen denaturation [15]. DSC has to reveal an elevation of the heat flow per unit mass during collagen denaturation in diabetic skin samples [16–17].

The protein collagen is the most predominant and most important protein of the skin. The existing types of collagens are characterized by a variety extend of triple-helical structure and a certain quantity of hydroxyproline. The structural variety of collagens is the result of the combination of tripple-helical domains with globular, non-helical domains. Type I collagen is the most interesting for industries utilizing collagen. The molecules of type I collagen form fibrils by longitudinal and lateral staggering with a typical cross-striation of 70-nm period. Fibrils form fibril bundles (elementary fibers), fibers and at last the fiber framework originates. Type I collagen has a basic structural unit—tropocollagen which is a molecular rod about 3000 nm in length, 1.4 nm in diameter, and 300000 molecular weight. The collagen is used in various ways, especially in some medical and pharmaceutical products. In particular, type I collagen is widely used as a raw material for medical devices owing to its low antigenicity and its ability to support cell adhesion and development. As examples of medical collagen application, it may be mentioned wound dressing for skin and eyes injures, bone and liver implants, and haemostatic dressings [18–20].

Collagen is widely used as a base material for a variety of drug carrier systems, and numerous studies have demonstrated its general biocompatibility and biodegradability. Focal points include collagenous lenses for ophtmalogic delivery and collagen sponges impregnated with antibiotics utilized in surgery to improve wound healing and to prevent infections like osteomyelitis [12].

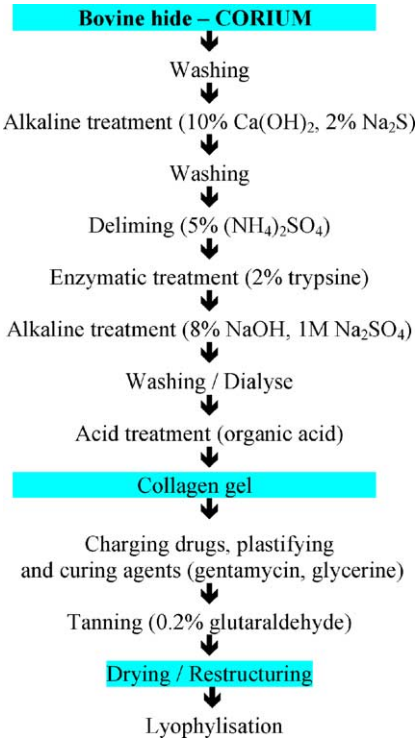
The present paper represents the first part of a complex research concerning to the development of collageneous membrane models which enable an optimum drug delivery in connection with the drug structure and trans-dermal usage. The main propose of the first part of this work is to apply mainly differential scanning calorimetry, pore size, permeability, and absorbtion measurments to study the thermal and structural changes caused by the antibiotic gentamycin in the type I collagen membranes. Such information may include: (a) thermal stabilization or destabilization of the membrane; (b) promote or retard the denaturation of the collagen membrane; (c) re-absorption properties of the collagen membrane; (d) permeability properties especially if data are combined with other experimental techniques like porosimetry; and (e) drug release properties. The provided information when coupled with other biophysical techniques (i.e. solid state NMR spectroscopy) shed more light on the possible use of a drug in collagen membranes for drug release purposes [21,22].

Pore size measurements may provide useful data regarding the collagen membrane micro-structure and morphology. Experimental data in connection with gas and liquid permeability results may also stress some useful information about the relationship between morphology and the controlled release capacity.

2. Experimental

2.1. Materials

Fibrous collageneous membranes were obtained by the procedure presented below which allows to obtain type I collagen gels. The chemical and enzymatic processes of this technology allow to extract more than 90% of structural fragment of native conformation collagen molecules or their polymerizates. The fibrous collagenous membranes were obtained by drying and using a restructurating procedure. The flow-chart for obtaining the fibrous collageneous membranes with antibiotic gentamycin is given below [23,24].



2.2. Methods

2.2.1. Differential scanning calorimetry

DSC technique was applied to study collageneous samples using a Perkin-Elmer DSC–7 calorimeter. An amount of 5–10 mg of the collageneous membrane with or without gentamycin was sealed into stainless steel capsules obtained from Perkin-Elmer. Thermograms were obtained on a Perkin-Elmer DSC-7 calorimeter (Nowalk, Connecticut, USA) [1–3].

The pore size measurements were carried out by the use of a Coulter Porometer II. This one possesses a 300–0.05 μm maximum pore size range and is based on the "wet-dry" measurement technique. The samples of 25 mm diameter were wetted in the Coulter Porofil, which is a filtrated fluorinated hydrocarbon (γ = 16 mN/m). The N_2 permeability was also determined by the use of the Coulter Porometer II, as the slope of the N_2 flow-rate versus pressure dependence. The water vapor permeability was performed according SREN ISO 14268/2003 [23].

3. Results and discussion

3.1. The effects of different concentrations of gentamycin on FM

Fig. 1 shows the effects of gentamycin at a range of 0.5–3% w/w in FM (AMATCOL). The top DSC scan on Fig. 1 shows the collagen matrix without antibiotic characterized by two broad peaks labeled as regions *T*m1 (25–75 °C) and *T*m2 (90–130 °C). Differences between the preparations with absence or presence of gentamycin are observed in both regions *T*m1 and *T*m2 (Fig. 1, left). Both regions *T*m1 and *T*m2 become less complex in peaking as the concentration of gentamycin increases. The incorporation of >1.5% w/w gentamycin results in almost identical thermal profiles. This shows that significant changes occur in the collageneous matrix when the concentration of gentamycin is raised between 0.5–1.5% w/w. More specifically the complexity of the region *T*m2 is simplified when gentamycin is incorporated at >1% w/w. The peak at 110 °C belonging to *T*m2 is shifted towards 120 °C when the concentration of gentamycin is

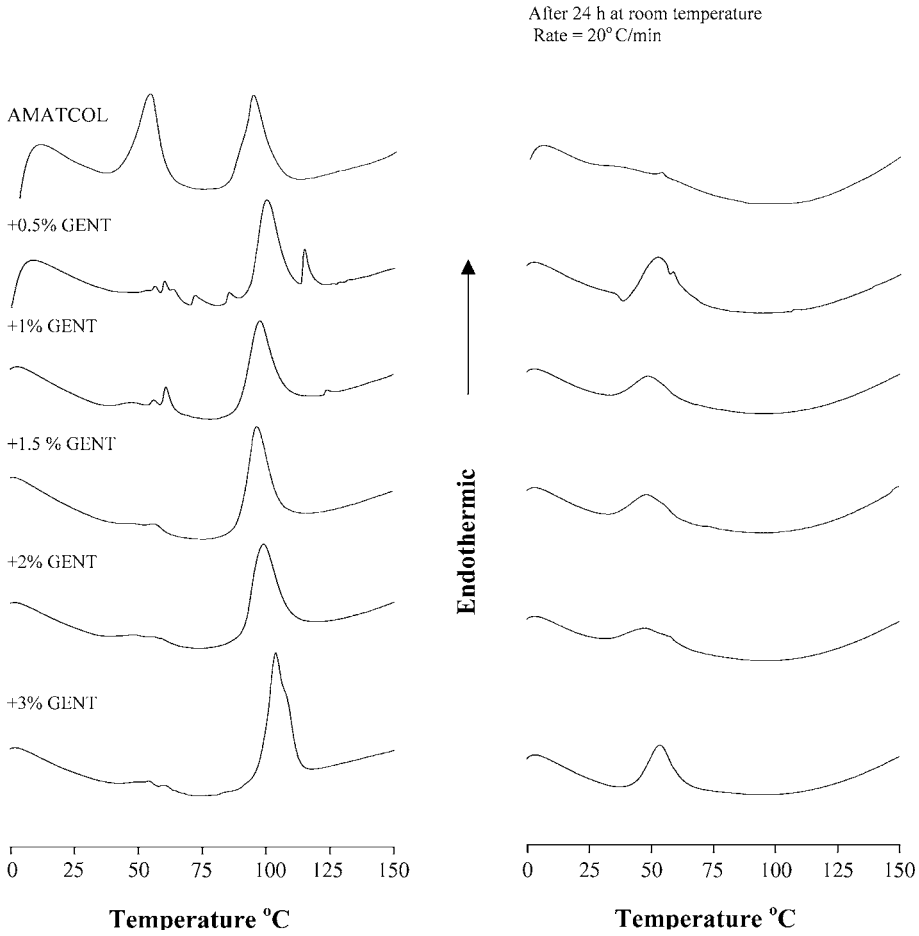


Fig. 1. DSC scans of FM using a rate of 20 °C/min type of collageneous membranes and different concentrations of gentamycin. DSC scans obtained (left) right after sealing with and (right) after a 24 h leaving the sample at room temperature.

Table 1
Diagnostic DSC parameters of the fibrous collageneous membrane alone and with different percentage of gentamycin after sealing and after 24 h equilibration time at room temperature

Sample	*T*m1		*T*m2	Tonset			$Tm_{1/2}$		ΔH_1 (J/g)		ΔH_2 (J/g)	ΔH_t (J/g)	
AMATCOL	54.3		94.4	44.3		88.7	10.2	9.9	17.2		17.5	34.7	
+0.5 GENT	60.3	72.2	100.0	58.6	70.7	93.6		8.6	3.2	0.7	20.2	24.1	
+1.0 GENT	61.0		97.9	58.9		89.7		10.0	4.0		18.0	22.0	
+1.5 GENT	56.9		96.8	49.1		90.5		8.9	1.4		19.1	20.5	
+2.0 GENT	56.2		99.0	40.1		91.4		10.0	1.9		19.8	21.7	
+3.0 GENT	54.3		103.8	48.7		98.5		8.2	1.8		21.1	22.9	
After 24 h equilibration at room temperature rate = 20 °C/min													ΔH_{tl}
AMATCOL	53.7			47.9		29.2				3.2		3.2	90.8
+0.5 GENT	52.7			41.0		14.3			13.0		0.02	13.02	46.0
+1.0 GENT	49.1			37.3		15.7				6.5		6.5	70.5
+1.5 GENT	48.9			36.6		17.9				6.6		6.6	67.8
+2.0 GENT	48.3			34.4		20.7				5.7		5.7	73.7
+3.0 GENT	53.7			44.1		12.1				9.1		9.1	60.3

1% w/w and disappears at higher concentrations. The samples were left 24 h at room temperature for equilibration after heated above 150 °C and DSC scans were obtained using a scanning rate of 20 °C/min (Fig. 1, right). Significant changes were observed between the preparation without drug and those containing different concentrations of gentamycin. ΔH of all preparations containing gentamycin had a higher value with maximum value observed in the preparation containing 0.5% w/w (see Table 1). ΔH_t is the summation of ΔH_1 and ΔH_2 corresponding to *T*m1 and *T*m2 regions. The percent decrease of ΔH (ΔH_{t1}) is inversely proportional to re-absorption rate and is the lowest when FM is without drug. The shape of the transition also varied according to drug concentration. For example, the preparation containing 3% w/w gentamycin showed the sharpest peak.

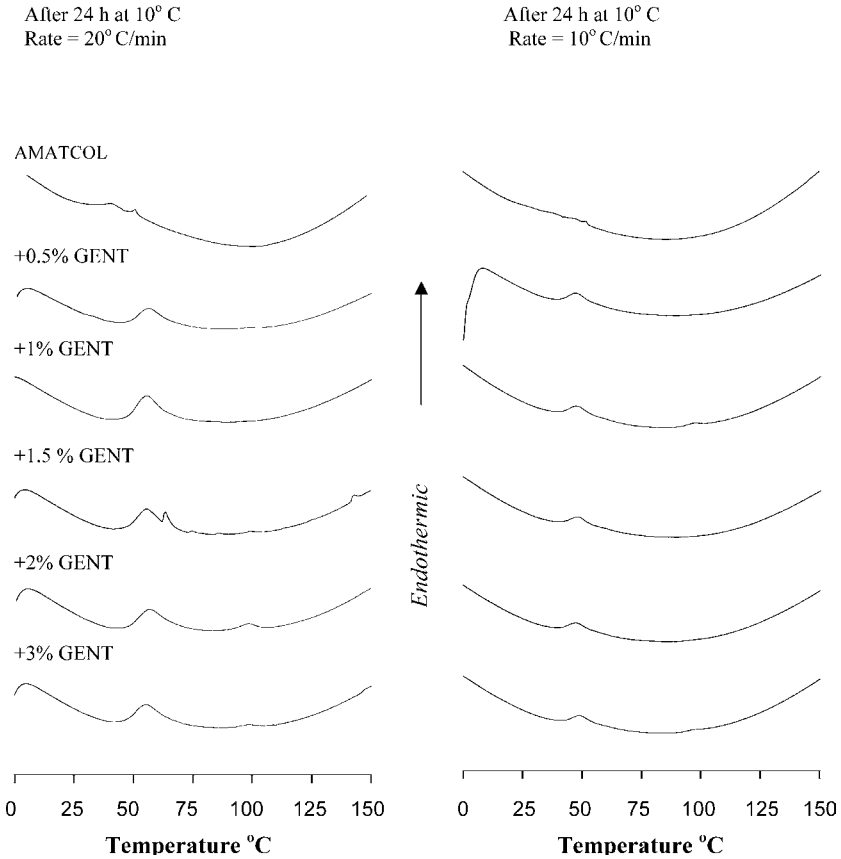


Fig. 2. DSC scans of FM using (left) a rate 20 °C/min and (right) a rate of 10 °C/min type of collageneous membranes and different concentrations of gentamycin.

Table 2
Diagnostic DSC parameters of the fibrous collageneous membrane alone and with different percentage of gentamycin after 24 h equilibration time at 10 °C using different scanning rates

Sample	*T*m	Tonset	*T*m1/2	ΔH (J/g)	ΔH_{tl}
After 24-h equilibration at 10 °C rate = 20 °C/min					
AMATCOL	53.0	34.5	20.5	2.6	92.5
+0.5 GENT	55.9	47.9	12.9	3.2	86.7
+1.0 GENT	55.7	47.5	12.1	5.0	77.3
+1.5 GENT	55.7	47.4	15.7	5.6	72.7
+2.0 GENT	56.5	47.7	13.6	4.4	79.7
+3.0 GENT	55.6	47.2	13.6	4.3	81.2
After 24 h at 10 °C rate = 10 °C/min					
AMATCOL	52.0	42.3	–	1.0	97.2
+0.5 GENT	47.6	40.9	9.3	3.3	86.3
+1.0 GENT	48.8	42.2	9.3	3.2	85.5
+1.5 GENT	48.5	42.0	9.3	2.7	86.8
+2.0 GENT	48.0	41.2	9.3	2.6	88.0
+3.0 GENT	48.7	42.7	9.3	2.4	89.5

3.2. The effect of scanning rate in FM containing different concentrations of gentamycin

FM without and with the incorporation of the drug were studied after leaving them in equilibration for 24 h at 10 °C using different scanning rates (see Fig. 2, left). Only *T*m1 region is eminent in the most thermograms. The most important feature in these DSC scans is that ΔH_{tl} is highest in the absence of drug as was already mentioned previously with the samples equilibrated at different temperatures (see Table 2). The scanning rate did not affect significantly the DSC thermograms (Fig. 2, right). Again ΔH_{tl} is highest in the FM membrane without drug.

3.3. Denaturation of FM

Fig. 3 shows the denaturation of matrices. This occurs at 244.2 °C when pure collagen matrix is used. When gentamycin is incorporated, the denaturation is shifted towards higher temperatures (261.8, 248.7, 257.8, 255.0, and 253.4 for ascending concentration of gentamycin 0.5, 1.0, 1.5, 2.0, and 3.0% w/w) indicating again that gentamycin stabilizes the collagen matrices. However, the increase of *T*m due to the presence of drugs is not linear and *T*max was observed when 0.5% w/w gentamycin was incorporated in FM.

3.4. Pore size, absorbtion, and permeability measurements

3.4.1. Pore diameters

The pore diameters for all preperations were measured (see Table 3). A concentration of gentamycin increase versus mean pore diameter is plotted in Fig. 4. As it can be observed the mean pore diameter of FM decreases as the concentration of gentamycin increases up to 2% w/w and starts to increase at higher concentration.

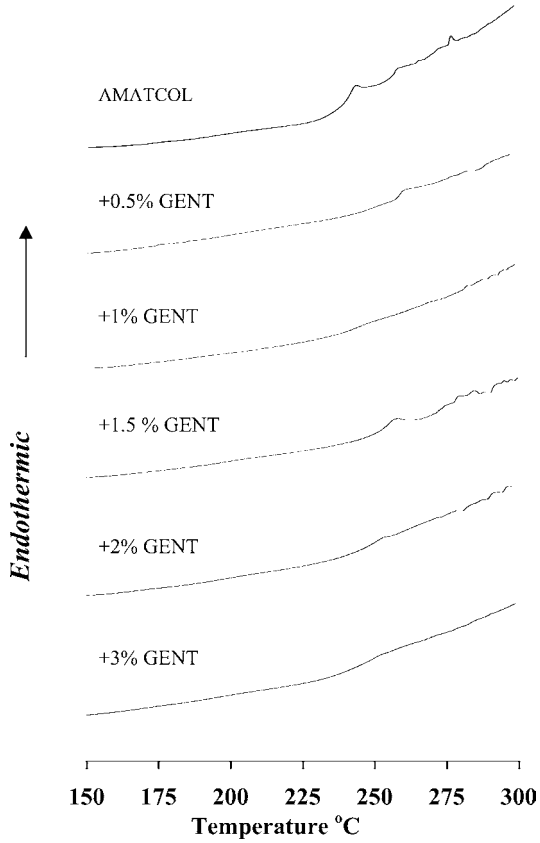


Fig. 3. DSC scans of FM using a rate 20 °C/min at a temperature of 150–300 °C where a denaturation of collageneous membrane happens.

3.4.2. Permeability studies

The permeability of the same samples in N_2 and water vapors were also measured (see Table 3). The permeability of nitrogen and water vapors as function of gentamycin's concentration are plotted in Fig. 5. The permeability of nitrogen versus increase concentration of gentamycin follows the same trend as the mean pore diameters. However, the permeability in water vapors starts from a low value, increases, and then reaches the same minimum when concentration of gentamycin is 2.0% w/w. Above 2.0% w/w, an increase in the permeability in water vapors is observed.

The absorbtion of water as a function of gentamycin's concentration is shown in Fig. 6 (see also Table 3). Again, a parallel trend was observed as in mean pore diameters and permeability of nitrogen measurements.

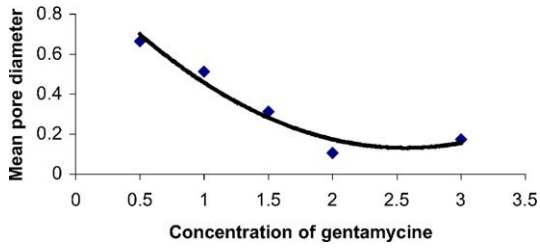


Fig. 4. Mean pore diameters of FM as a function of gentamycin's concentration.

Table 3
Physical properties of fibrous collageneous membranes with different percentage of gentamycin

Gentamicyne concentration	Concentration of glycerol (agent of plasticization)	pH	Water absorption after 2 h (%)	Water vapor permeability mg/24 h	Pore size (μm)	Nitrogen permeability (L/cm^2 min bar)
0.5	0	7.2	220	441	0.664	4.360
1	0	7.2	213	493	0.514	2.618
1.5	0	7.2	210	477	0.314	1.845
2	0	7.2	178	420	0.105	0.67
3	0	7.2	200	444	0.174	0.610

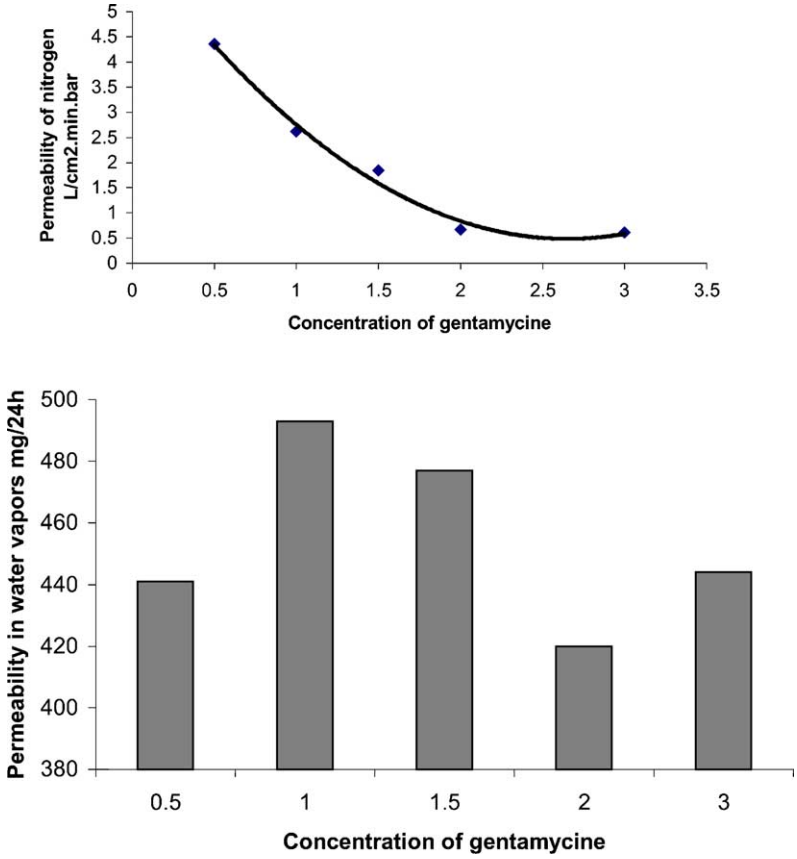


Fig. 5. (Top) permeability of nitrogen and (bottom) permeability of water vapors as a function of gentamycin's concentration.

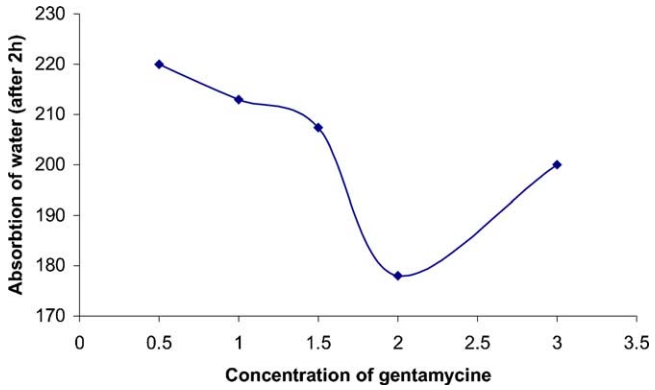


Fig. 6. Absorbtion of water as a function of gentamycin's concentration.

4. Conclusions

Significant conclusions can be derived from the use of DSC and porosimetry studies using FM membrane in our studies: (a) the 35–70 °C region endothermic effect attributed to the collapse of the triple-helical domain of collagen is affected by the presence of gentamycin. In particular, the thermal effect consisting of several peaks of the collageneous membrane with low percentage of drug is simplified to a broad peak when higher concentrations are used. This is not a surprising result and it clearly indicates that gentamycin interplaces between collagen triple helices. Its topography probably affects the hydrogen bondings between the triple helices or new hydrogen bondings are formed between gentamycin

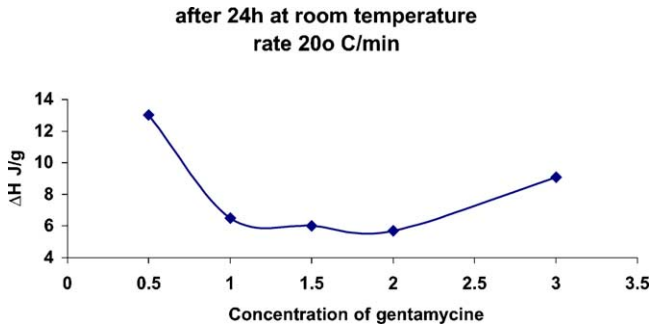


Fig. 7. ΔH as a function of gentamycin's concentration.

and aminoacids generating the triple helix of collagen; (b) the endothermic peaks due to dehydration of collagen membrane at temperatures of 90–120 °C are also affected by the presence of gentamycin. This region consists of two peaks at low percentage of gentamycin. At higher percentages, the peak near 120 °C decreases in intensity and finally disappears. This result indicates that gentamycin affects the dehydration pattern of collageneous membrane; (c) the thermal stability of collageneous membranes is increased by the presence of antiobiotic gentamycin as it is seen by examining the region between 150–300 °C; (d) ΔH parallels with mean pore diameter, permeability of nitrogen, and absorbtion of water as a function of gentamycin concentration (Fig. 7). Thus, maximum re-absorbtion of water occurs when gentamycin concentration is 2.0% w/w; and (e) the scanning rate affects ΔH and shape of peaks. However, the overall trend of percent decrease of ΔH_t does not depend on the scanning rate. This is a useful parameter to establish if different experiments are performed using different scanning rate.

Gentamycin is a largely employed antibiotic in various skin injuries (burns or infected sores) or bone diseases, where the wounds are required to be kept sterile. The collagenous membranes prepared according to the flow-chart presented in the paper have not been tested as trans-dermal drug delivery membranes. These membranes containing various bioactive substances have been applied in situ as implants and tissue grafts. Delivery of gentamycin contained in collagenous membranes depends on the morphological structure of macro-, micro-, and nano-porous structure resulted from the freeze drying of the collagen gel containing gentamicin. Collagen structure, restored from the gels by drying and chemical cross-linking, acts on membrane morphological structure and thermal, physical–chemical, mechanical, and micro-biological stability. These characteristics also act on the trans-dermal delivery level.

We are currently using other types of membranes in our studies in an effort to compare their physical and thermal properties in absence and presence of drugs. A comparison of their properties will be sought in order to reveal the optimum matrix of incorporation of gentamycin and other bioactive drugs. In addition, these studies will give valuable information on the properties of drugs in collagen devices. This information may find application in the biotechnology of drug delivery system.

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