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# MEASUREMENT AND INTERPRETATION OF WETTING PROPERTIES OF NEW COLLAGEN-SILICATE BIOMATERIAL

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New nontoxic, biocompatible, collagen-natural silicate membranes were prepared. The morphology of nanostructured materials (partially exfoliated, exfoliated or intercalated) depends on the preparation method. The incorporation of a bioactive drug (gentamycine) in the silicate before the membranes casting induces an intercalated nanostructure. The used silicate was montmorillonite, a purified natural bentonite (Bentonite of Chioar Valley Roumania). Through contact angle measurements of membrane/air and membrane/polyethylene support, the surface tension ($\gamma_s$), electron – acceptor ($\gamma_s^+$) and electron donor ($\gamma_s^-$) surface components of biomaterial samples were calculated using the Lifshitz-van der Waals/Lewis acid-base interaction model. The dynamic contact angle was used to determine the surface porosity, partial soaking and the rate of water diffusion in the membrane, which was found to reflect the nanostructured morphology.
The nanocomposite morphology was studied using X-Ray diffraction and SEM methods whose results are in good agreement with the wettability data. The new membranes are promising transdermal biomaterials.

## INTRODUCTION

Measurement and interpretation of biomaterial wetting properties are a modern approach in interfacial chemistry of biomaterial science.

In order to use a material for biomedical purposes its bulk and surface properties are both important to be known, especially interfacial behaviour with aqueous environment. The water contact angle and water absorbency gave valuable information in the evaluation of the biocompatibility with living body. The surface free energy $\gamma$ of the material is correlated to the contact angle that is directly related to its wettability and other physico-chemical properties. Recently the $\gamma_S$ for montmorillonite, the used silicate in the present paper, was determined based on liquid/solid interaction and was found to have the real value 205.066 ±2.764 mJ/m$^2$.[1]By wettability one find a possibility to predict the results in a hydrated state from the surface tension results of the dry gel membranes.[2,3] In the category of soft materials which posses low interfacial energy and excellent biocompatibility,[4] the collagen, collagen silicate composite are one of the best.[5] Some collagen sheet with gentamicyn sulphate were found to be very useful in first and second degree burns.[6] The wettability method is a convenient way to acquire an inside for the surface properties of biomedical materials and the swelling behaviour. This method is suitable to detect the presence of roughness in the surface as well. Sometimes this is a positive quality because the roughness of a material induces a better cell attachment.[7,8] Liquid and vapour water absorption and the wettability of the membranes are important factors when the release of drugs from the biomembrane is followed.

The aim of this research is the study of surface properties of new collagen-silicate membranes with different nanostructures able to embed and release a bioactive drug such as gentamicin sulfate. A purified and inexpensive silicate with applications in cosmetic and food industries, bentonite from Chioar Valley Roumania, a good candidate for transdermal pharmaceutical applications was used.

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## MATERIALS AND METHODS

Gel of collagen (COL) with native structure, extracted from bovine skin, with molecular weight 300.000 Da at pH 2.6 was obtained from National Institute of Research and Development for Textile and Leather, Bucharest, Roumania. Sodium montmorillonite (MMT) obtained by bentonite purification (Bentonite of Valea Chioarului-Roumania-BVC) with basal spacing $d_{001}$: 15.1Å and the inorganic residue determined by TGA at 700 °C, $R_{700}$: 87.7 % and gentamicin sulfate (GENT) from pharmaceutical injectable ampoules of 40mg/1ml were used.

Test liquids: deionised water, α –bromonaphthalene, dimethyl sulfoxide (Sigma products) were used as received.

### Preparation of nanocomposite membranes

The collagen/layered silicate nanocomposites (BVC MEMs) were obtained by inserting the biopolymer into the galleries, between the MMT layers, using solution intercalation method. For the compatibilization with collagen, the silicate surface was modified with: collagen (at a silicate/collagen ratio of 1/0.3), gentamicin (at a silicate/ gentamycine ratio of 1/0.055) and with collagen and gentamicin mixture (at a silicate/mixture ratio of 1/0.355). Before dispersing the layered silicate was swelled 1% in deionised water for 48h.

For nanocomposite films preparation the mixtures were casted onto polyethylene support, (PE). The films were dried for 48 h, in an air-drying apparatus at 25 – 28°C. The following formulations were obtained (Table 1):

*Table 1*

BVC MEMs collagen-silicate membrane formulations

| Membrane code BVC MEM | Membrane basic composition | | | Modifying agents for MMT | | Membranes Morphology (X -Ray) |
|---|---|---|---|---|---|---|
| 1 | COL | 20 % MMT | - | - | GENT | intercalated |
| 2 | COL | 20 % MMT | - | COL | GENT | partially exfoliated |
| 3 | COL | 20 % MMT | GENT | COL | - | exfoliated |
| 4 | COL | 20 % MMT | GENT | - | - | partially exfoliated |
| 5 | COLcontrol | - | - | - | - | Fibrilar |
| 6 | COLcontrol | - | GENT | - | - | Fibrilar |
| 7* | COL | 20 % MMT | GENT | - | - | exfoliated |

* For BVC –MEM 7 membrane (collagen + gentamicin + 20 % unmodified MMT) the silicate was obtained from the supernatant solution, resulted after swelling 1% silicate in deionised water for 48h

### Wettability

Wettability studies were conducted according to the liquid-solid contact angle method. A tiny drop of a liquid with known properties (Table 2), is gently deposited on the membrane surface and the contact angle (θ) is measured during a time interval from several seconds to twenty minutes. The image of the drop profile is recorded by video enhanced microscopy VEM. The volume of the liquid droplets was between 3-5 μL. To avoid any effect of drop size on the measured contact angles at least six liquid drops were applied on different sites at 20-21$^0$ C on the both surfaces (air and polyethylene). The advancing ($\theta_a$) and receding ($\theta_r$) contact angle were measured in order to observe the contact angle hysteresis.

The permeability to water of the dry membranes was measured from the variation of droplet volume (8 μl) in time by the contact angle values.

### X-ray diffraction

The morphology of the nanocomposites was determined by X-ray diffraction (XRD). The patterns were automatically recorded at small angles (2θ=2÷30°), on a DRON-2, 0 X-ray diffractometer with horizontal goniometer; $CoK_\alpha$ the radiation source (λ = 1,789 Å) was used, filtrated with Ni for $K_\beta$ component removing, in Bragg-Brentano system (by reflection).

### Scanning Electron Microscopy (SEM)

SEM images of BVC MEMs 3,4 and 6 were taken with a FEI Quanta 200 microscope. The membranes were observed in high vacuum, without any metal coating prior to analysis.

### Video Enchanced Microscopy (VEM)

The VEM method was used in order to measure the drops profile and follow the dynamic contact

angle aspect of both surfaces (air and PE) with a JVC TK-C920/TK-C921 video camera in time.

## RESULTS AND DISCUSSION

The experimental results are shown in Tables 1-2 and Figs. 1-5.

The θ values depend on chemical composition, porosity and roughness of the surface. The studied membranes might have all those properties. The membrane/PE surface gives a water contact angle, $\theta_{W,}$ values higher than $\theta_W$ on membrane/air surface, for all the samples (Fig. 1). This difference might be due to the roughness or porosity of the BVC MEMs/interface or to the presence of different components in the interfacial layers, a sort of chemical heterogeneity due to some patchy like energetic structure of the surface, or both. Due to the presence of roughness on surfaces, the values of $\theta_W$ are diminishing when the real value of $\theta_W$ are smaller than 90°.[9] In case of our experimental data, $\theta_{W/air} < \theta_{W/PE,}$ we can draw the conclusion that the BVC MEM/PE interface is smoother than the BVC MEM/air interface for all the studied membranes. This conclusion was supported by the absence of the contact angle hysteresis at the BVC MEM/PE interface and by the SEM images (Fig. 4).

*Table 2*

The surface energy components (mJ/m2) of collagen-silicate membranes (completeYoung eq.1)

| | Materials | Surface free energy* ($mJ/m^2$) | | | | |
|---|---|---|---|---|---|---|
| | | $\gamma^+$ | $\gamma^-$ | $\gamma^{AB}$ | $\gamma^{LW}$ | $\gamma^{TOT} = \gamma^{AB} + \gamma^{LW}$ |
| Liquids | α-bromonaphthalene | 0 | 0 | 0 | 43.5 | 43.5 |
| | dimethyl sulfoxide | 0.5 | 32.0 | 8.0 | 36.0 | 44.0 |
| | water | 25.5 | 25.5 | 51.0 | 21.8 | 72.8 |
| Membranes (air interface) | BVC MEM 1 | 1.0 | 25.4 | 10.0 | 43.5 | 53.5 |
| | BVC MEM 2 | 2.8 | 20.7 | 15.5 | 43.5 | 59.0 |
| | BVC MEM 3 | 0.2 | 14.5 | 3.5 | 43.5 | 47.1 |
| | BVC MEM 4 | 1.05 | 48.4 | 14.2 | 43.5 | 57.7 |
| | BVC MEM 5 | 0.8 | 21.7 | 8.5 | 43.5 | 52.0 |
| | BVC MEM 6 | 1.6 | 58.9 | 19.8 | 43.5 | 63.3 |
| | BVC MEM 7 | 0.02 | 32.02 | 1.6 | 42.6 | 44.2 |
| Membranes (PE interface) | BVC MEM 1 | 1.5 | 8.1 | 7.0 | 43.5 | 50.5 |
| | BVC MEM 2 | 2.8 | 38.1 | 20.7 | 43.5 | 64.2 |
| | BVC MEM 3 | 0.5 | 3.3 | 2.7 | 43.5 | 46.2 |
| | BVC MEM 4 | 1.4 | 20.4 | 10.6 | 43.5 | 54.2 |
| | BVC MEM 5 | 0.08 | 5.7 | 1.3 | 43.5 | 44.8 |
| | BVC MEM 6 | 2.8 | 55.0 | 24.8 | 43.5 | 68.3 |
| | BVC MEM 7 | 0.0 | 6.6 | 0.2 | 43.5 | 43.7 |

* The surface free energy ($mJ/m^2$) is also referred as the surface tension (mN/m).

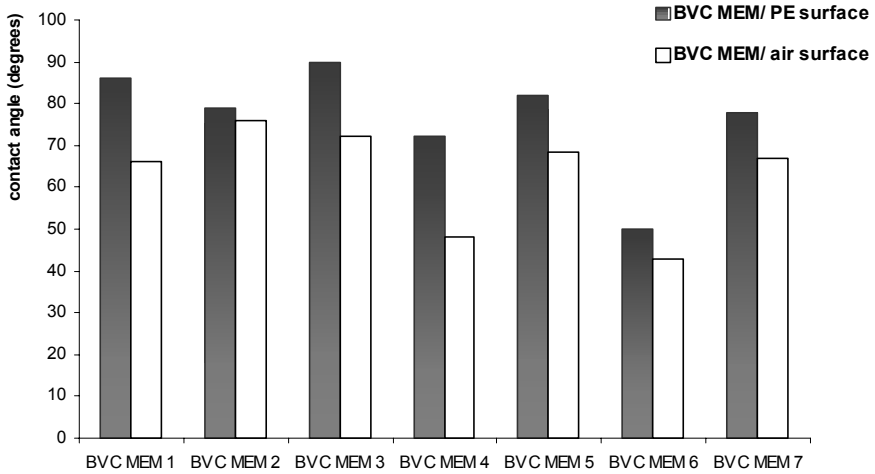



Fig. 1 – Water contact angle $\theta_W$ on collagen-silicate membranes BVC MEMs/air and MEMs/PE at 20-21°C.

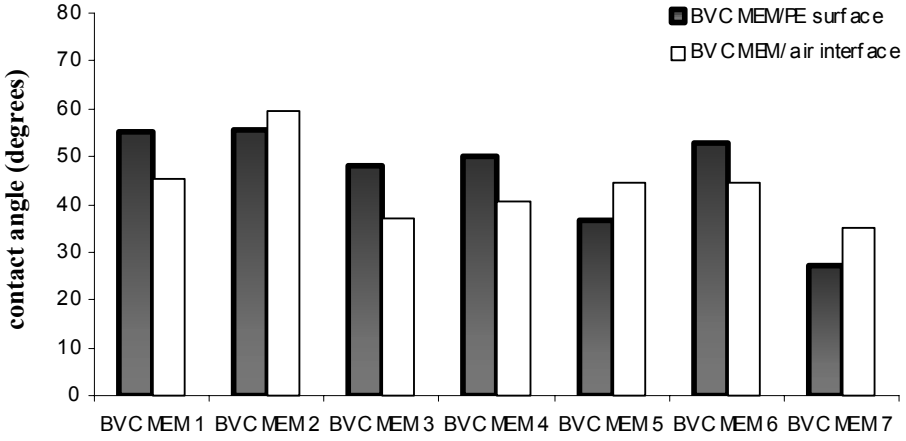



Fig. 2 – Contact angle $\theta_{DMSO}$ on collagen-silicate membranes (BVC MEMs) at the air and PE interfaces at 20-21 $^{o}$C.

The contact angle hysteresis defined as $\theta_a - \theta_r$, was observed only on the BVC MEM/air interface and even then was very small. Due to the fact that BVC MEM/PE membrane side is smoother than BVC MEM/air side for all the samples, it is better to compare the membranes on the smoother side especially when talking about chemical composition induced by the preparation method. [10]

A complete characterization of surface properties of new collagen-silicate membranes was studied in this paper by means of complete "Young equation" (eq.1)

$$(1+\cos\theta)\gamma_L^{TOT} = 2(\gamma_S^{LW}\gamma_L^{LW})^{1/2} + 2(\gamma_S^{+}\gamma_L^{-})^{1/2} + 2(\gamma_S^{-}\gamma_L^{+})^{1/2} \quad (1)$$

The apolar Lifshitz – van der Waals ($\gamma^{LW}$) interactions including the dispersion (London), orientation (Keeson) and the induction (Debye) interactions in condensed state, and the polar, Lewis acid-base ($\gamma^{AB}$) components are additive, while the Lewis acid-base electron acceptor ($\gamma^{+}$) and electron donor ($\gamma^{-}$) are not additive. That is why we determined θ with three different liquids, α–bromonaphthalene, dimethyl sulfoxide and water, liquids that are completely characterized by their $\gamma_L^{LW}$, $\gamma_L^{+}$, $\gamma_L^{-}$ (Table 2) [11] and introduce the data in eq. 1.

The results are shown in Table 2 and Figs. 1 and 2. We mention that the θ values using α-bromonaphthalene as test liquid are not presented because they were not significant ($\theta < 10^0$) and the calculation of $\gamma^{LW}$ gave very similar values for all the samples.

The complete Young equation (eq. 1) was applied to natural and synthetic solid surfaces which emphasize that many such surfaces have a monopolar $\gamma^{-}$ character: most proteins, polysaccharides, sucrose, cellulose esters and even aromatic compounds such as polystyrene on account of the donor behaviour of electrons. [12]

Colloidal stability of the gels before and during membranes casting is related with $\gamma^{AB}$ repulsive interaction based on the large surface electron donicity ($\gamma^{-}$) of the nanocomposite components. [13] In Table 2, $\gamma^{-} > \gamma^{+}$ for all composite silicate membranes, which shows the presence of silicate and collagen in the surface. Flocculation processes are caused by attractive $\gamma^{AB}$ (hydrophilic) interactions, and the Van der Waals $\gamma^{LW}$ forces only make a secondary contribution. [14] Data obtained with DMSO as test liquid give information concerning the difference of contact angles dependence of chemical surface composition more than roughness. This test liquid has a surface energy $\gamma^{TOT}$ preponderantly given by $\gamma^{LW}$ (Table 2); BVC MEM 2, 5, 7 have $\theta_{DMSO}$ values decreasing in this order while in case of water as test liquid $\theta_W$ values are closer to each other. The θ values measured with DMSO as test liquid (Fig. 2) are smaller than θ values with water for all the samples because DMSO is interacting with the membranes only by $\gamma^{LW}$.

The gentamicin presence in the surface induces a hydrophilicity increase ($\theta_{W\ BVC\ MEM6} < \theta_{W\ BVC\ MEM\ 5}$).

Our experimental data display a big difference at BVC MEM/air interface between the samples: BVC MEM 2 and BVC MEM 3 have $\theta_W$ values higher than BVC MEM 5 (collagen membrane) while BVC MEM 4 and BVC MEM 6 have $\theta_W$ values lower than BVC MEM 5. For BVC MEM 4

and BVC MEM 6 the difference might be given by the presence of micropores. The bigger the surface pore fraction on surface, the higher the contact angle value, which means that the wettability of the porous membrane is modified. This is a way to control the biomaterial's surface wetting properties by surface porosity and microscopic "spikes".[15]

The wettability data by dynamics of water droplet absorption can reveal the morphology of the membranes.

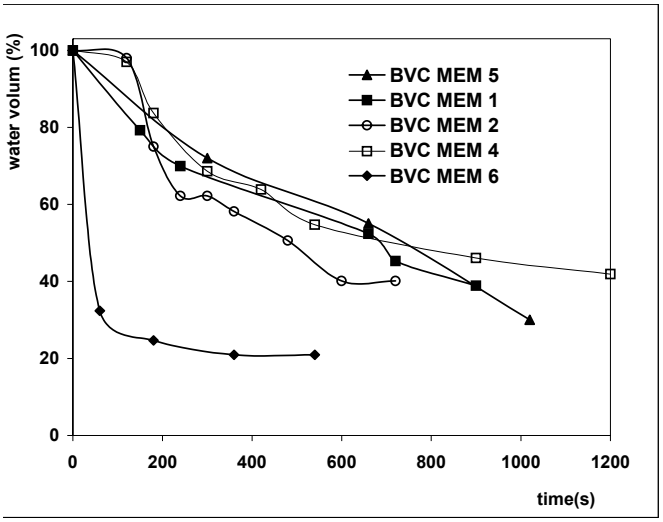



Fig. 3a – Variation of water droplets' volume (in percentages) with time for membranes BVC MEM 5,1,2,4,6 (only some experimental values are represented in this figure).

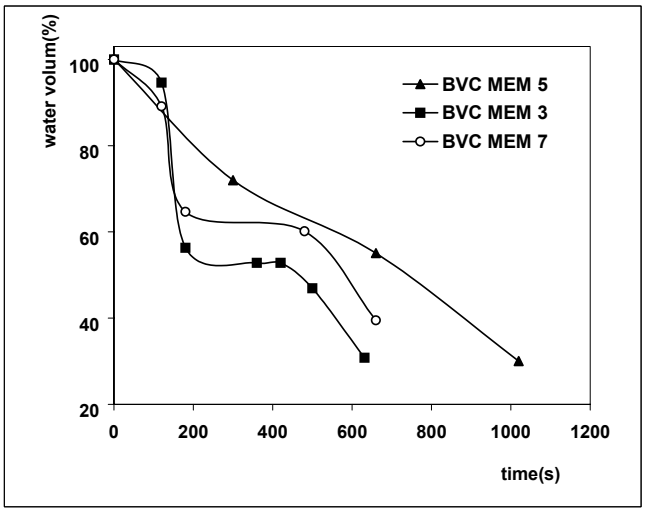



Fig. 3b – Variation of water droplets' volume (in percentages) with time for membranes BVC MEM 5,3,7 (only some experimental values are represented in this figure).

In Figs. 3a and 3b are not represented the real experimental data. In order to eliminate the subjective influence of drop's size we expressed the droplets' volume in the percentages. We follow how the droplet volume changes in time or in other words the rate of water diffusion into membranes. On both figures the BVC MEM 5 is represented since this is a pure collagen membrane. One can see two types of curves: a smooth curve (BVC MEM 1,5,6) and a step one (BVC MEM 2,3,4,7).

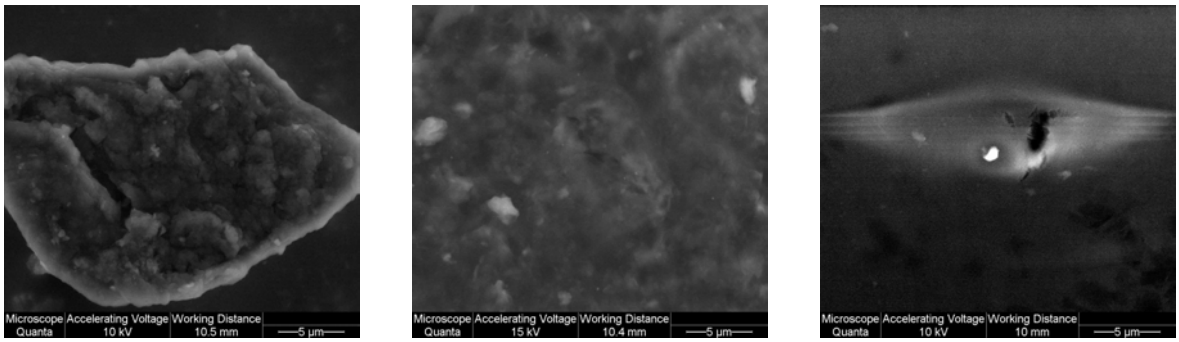
The aspect of the step curve may be correlated with the morphology of the nanocomposites, which presents an exfoliated and partial exfoliated structure. The BVC MEM 1 with an intercalated structure has the water absorption curve similar to collagen membranes. This behaviour demonstrates the influence of the preparation method.

Water vapour absorption (data not shown) follow same trend as water droplets absorption. The maximum water vapour absorption (after 70 h) is diminishing in the following order: BVC MEM 5> BVC MEM 6> BVC MEM 4& BVC MEM 1>> BVC MEM 3>> BVC MEM 2.

The SEM images for BVC MEM 3 show a rough and porous surface. BVC MEM 4 is less porous but rougher, because the MMT wasn't modified prior to membrane preparation. BVC MEM 6, Fig. 3a, presents a fibrous structure without high roughness and porosity because the presence of gentamicin sulfate generates a more hydrophilic surface correlated with big values of electron donor surface component (55 mJ/m$^2$) and the polar, Lewis acid-base, $\gamma^{AB}$ (24.8 mJ/m$^2$), when compared with pure collagen membrane BVC MEM 5 with electron donor surface component (5.7 mJ/m$^2$) and $\gamma^{AB}$ (1.3 mJ/m$^2$) (Table 2).

X-ray data show the different morphologies induced by different preparation method. From those data (Table 1) and from water diffusion data we draw the conclusion that for the intercalated morphology (BVC MEM 1) we have a smooth type of curve, for partially exfoliated (BVC MEM 2) and exfoliated structure (BVC MEM 3) we notice a step curve. All those different behaviours are influenced by the formulation method.

On VEM images for BVC MEM 3 on the smooth surface (PE interface) was observed a homogenous surface with white milky collagen spots, and on BVC MEM 4 on PE surface a homogenous appearance with grey silicate particles and collagen spots. On BVC MEM 1 there are lots of non-homogeneities of different geometries and long collagen fibbers.




**(a)** **(b)** **(c)**

Fig. 4 – SEM images of BVC MEM 3 (a) BVC MEM 4 (b) and BVC MEM 6 (c) at accelerating voltage 10 kV working distance 10 mm; 5 μm for (a,b,c).

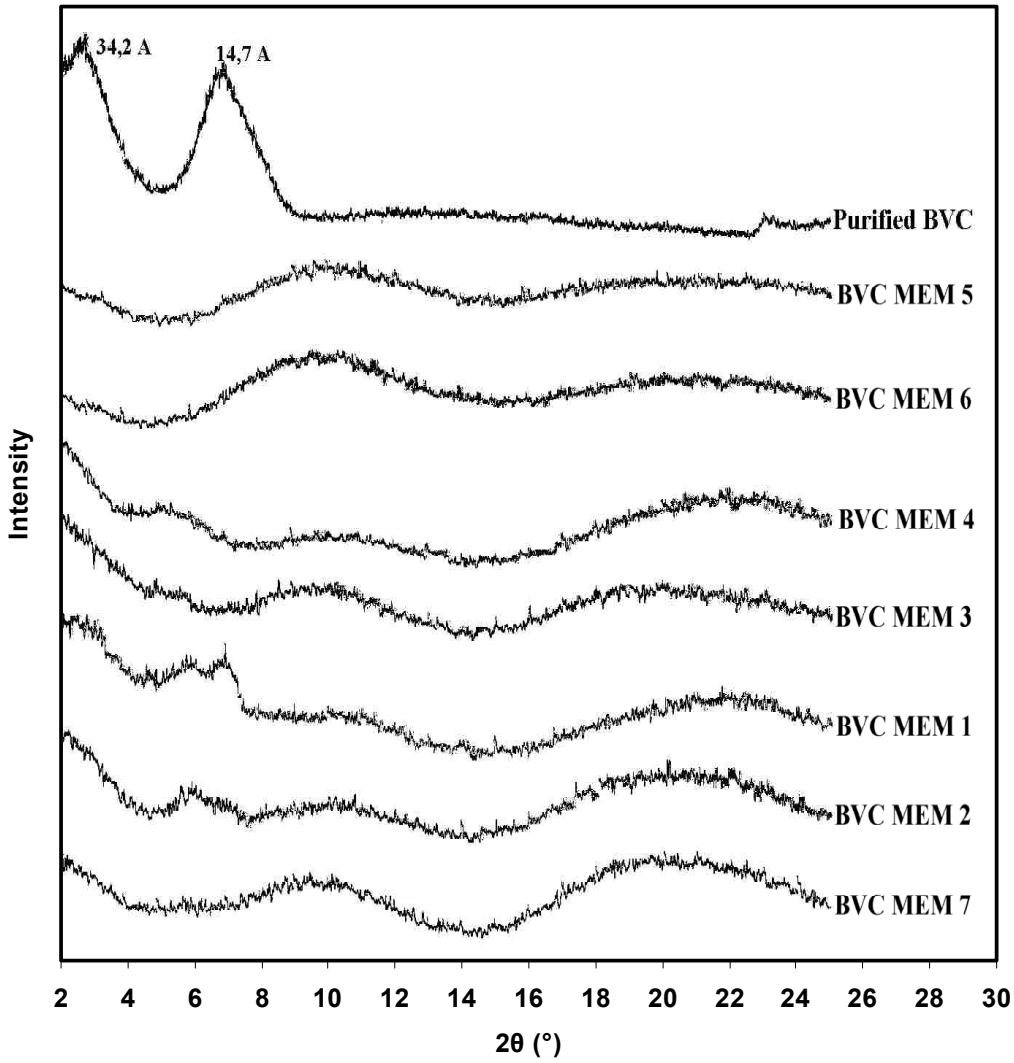



Fig. 5 – X-Ray results for the BVC MEMs as defined in Table 1.

BVC MEM 6 has been proven from all the studies to be the homogeneous and less rough membrane. From SEM images (Fig. 4), water diffusion data (Fig. 3a), VEM microscopy and contact angle data (Fig. 1) we draw the conclusion that BVC MEM 6 is a very homogeneous membrane.

The classification of water absorption rate is a reflection of preparation method that on its turn determines the membranes' morphology.

The wettability results correlated with the X-Ray and SEM data recommend this type of silicate/collagen membrane as drug delivery system for sustained release of gentamicin sulfate. This is in agreement with previous studies on matrices.[16]

## CONCLUSIONS

In this study the wetting properties of some new collagen based nanocomposite membranes, with different nanostructure of biomaterials (intercalated, partial exfoliated, exfoliated) were measured and discussed.

By contact angle method, surface energies, polar ($\gamma^{AB}$ ), van der Waals ($\gamma^{LW}$ ), roughness and porosity were measured and computed. A large surface electron-donor ($\gamma^{-}$ ) and an important $\gamma^{AB}$ polar surface energy were found for all nanocomposites. These properties may assure a good biocompatibility (important for uses as controlled release systems) and collagen gel stability during drying process.

The wettability method by dynamic of water droplet absorption revealed the nanostructure morphology of the new membrane, confirmed by X-ray diffraction and SEM data.

The relative simple and rapid wettability method gives us both the morphology and surface properties of the studied membranes.

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