MODELLING MESOSCALE DIFFUSION PROCESSES IN A BIOARTIFICIAL MEMBRANE

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*Acest studiu descrie eforturile noastre curente de a stabili un protocol prin intermediul abordării "bead-spring" implementată în Dinamica Disipării Particulelor (DPD) pentru investigarea difuziei Gentamicinei prin membrane polivinil alcool (PVA)-Chitosan. Modele computaționale la scală mezo de 10*10*10 unități reduse (r.u.) au fost implementate şi echilibrate în 100000 paşi prin intermediul DPD şi profilul eliberării Gentamicinei a fost examinat. Membranele PVA-Chitosan au fost realizate şi investigate în ceea ce priveşte structura morfologică şi biocompatibilitatea materialului. Materialul prezintă o distribuție omogenă a lanțurilor polimerice şi nu este citotoxic. O concordanță bună a fost găsită între rezultatele experimentale şi cele computaționale în ceea ce priveşte difuzia Gentamicinei şi morfologia materialului.*

*This study describes our current efforts to establish a protocol based on bead-spring approach encoded in Dissipative Particle Dynamics (DPD) to investigate Gentamicin diffusion through Chitosan-PVA membranes. Chitosan-PVA-Gentamicin mesoscale computational bulk models of 10*10*10 reduced units (r.u.) were implemented and equilibrated in 100,000 steps by means of DPD calculation, and Gentamicin release profile was studied. Chitosan-PVA membranes were obtained and investigated concerning material structural morphology and biocompatibility. The material displays a homogeneous distribution of polymeric chains and is not cytotoxic. An good agreement was found between experiments and simulations concerning both Gentamicin molecule diffusion and material morphology.*

Keywords: dissipative particle dynamics, drug release, *in vitro* indirect cytotoxicity tests, bioartificial polymeric material

1. Introduction

The diffusion in polymer membranes plays an important role in various chemical and biochemical processes and takes place at different time and length scales. Depending upon the spatial dimensions of the system and on the properties

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under investigation, computer modeling of such a process can range from forcefield based molecular dynamics (MD) to mesoscale simulation methods [1-3]. To understand and optimize the diffusion in polymer membranes, various models based on the free-volume concept have been successfully set-up [1,3-5]. However, inhomogeneous aspects such as migration of molecules, phase separation of polymer and diffusion of medium or large molecules, become important. Such phenomena are beyond atomistic models, and therefore, the development of novel methodology is required in order to extend the corresponding research area. These phenomena are crucial when the polymer membranes are evaluated for drug delivery systems. Delivery devices are polymer membranes, with the drug either dissolved in, or emulsified with the polymer, and have the scale of phenomena in the order of μm. Therefore, a coarse-grained model seems to be an appropriate approach to this problem. Mesoscale simulations have traditionally been used to investigate evolution of morphology in polymer systems, melts and blends [4-5]. Dissipative Particle Dynamics (DPD) is one of most known mesoscale simulation approach; the polymer chains are treated as coarse-grained bead-spring models. DPD was introduced in 1992 [6] in an attempt to go beyond the limitations of atomistic MD simulations, whilst retaining some molecular detail including the hydrodynamic interactions. Since then, the application of DPD technique has been extended to study microphase separation of polymeric mixtures, dynamics of an oil droplet near a hard surface in shear flow, self-assembly of a planar membrane, aggregation of surfactants onto a polymer, colloidal motion in a solvent, and rupture of a planar membrane patch by incorporation of nonionic surfactants [2, 5-7].

In the field of drug delivery systems, there is a growing demand for material which would reduce the incidence of complications in patients, and releasing the active agent is constant over a long period or might be triggered by environment or other external events. These aspects such as, biocompatibility and controlled drug delivery, occur when a polymer, whether natural or synthetic or even a blend of them, is judiciously chosen and combined with drugs or other active agents.

Bioartificial polymeric materials represent a new class of polymeric materials; these are blends of synthetic and natural polymers, designed with the purpose of producing new materials with enhanced properties for application as drug delivery systems [8]. Bioartificial polymeric materials were prepared as films or hydrogels using poly(vinyl alcohol) (PVA), poly(acrylic acid), poly(methacrylic acid) as synthetic components, and collagen, gelatin, chitosan, hyaluronic acid and dextran as biological components [8-10]. It was found that these materials gather the superior mechanical properties related to synthetic component and excellent biocompatibility related to the biocomponent [10]. Among the many synthetic polymers available for biomedical applications the

present study is addressed to PVA, which is one of the most widely used, due to its biocompatibility and chemical versatility and as biopolymer Chitosan. Chitosan, [(1→4)-2-amino-2-deoxy-β-D-glucan] is a natural aminopolysaccharide which has attracted much attention because of its good biological activity, biocompatibility and biodegradability [10].

In this study, a DPD approach was employed to investigate the Gentamicin distribution and diffusion in the PVA-Chitosan polymer membranes, and is coupled with an experimental approach used to produce PVA-Chitosan membranes and investigation concerning material morphology and cytotoxic activity.

2. Computational and experimental procedure

2.1. DPD mesoscale simulation method

DPD simulations consider materials a set of beads interacting by specified forces and whose dynamical evolution is governed by Newton's laws:

$$\frac{dr_i}{dt} = v_i, \qquad \frac{dv_i}{dt} = \frac{f_i}{m_i} \tag{1}$$

where r_i, v_i, f_i and m_i are the position vector, velocity, total force and mass, respectively, on the ith bead. All bead masses are assumed to be equal and are set equal to unity for simplicity. Each bead is subject to three non-bonded forces from its neighbours: a conservative force (F_{ij}^C) interaction which is linear in the bead-bead separation; a dissipative force (F_{ij}^D) proportional to the relative velocity of two beads, and a random force (F_{ij}^R) between a bead and each of its neighbours. The interaction between two particles can be written as the sum of these forces:

$$f_i = \sum_{i \neq j} \left(F_{ij}^C + F_{ij}^D + F_{ij}^R \right) \tag{2}$$

where, the sum is over all beads within a cutoff radius r_c of the ith bead. The conservative force is a repulsion acting along the line of centers, and is given by:

$$F_{ij}^C = \begin{cases} a_{ij}\left(1 - r_{ij}\right)\hat{r}_{ij}, & r_{ij} < 1 \\ 0, & r_{ij} > 1 \end{cases} \tag{3}$$

where, a_{ij} is a repulsion parameter between particle *i* and *j*, r_{ij} is the magnitude of the bead-bead vector r_{ij}, and $\hat{r}_{ij}$ is the unit vector joining beads *i* and *j*. The dissipative force F^D is proportional to the relative velocity of two beads acting to reduce their relative momentum:

$$F_{ij}^{D} = \begin{cases} -\gamma\omega^{D}(r_{ij})(\hat{r}_{ij}v_{ij})\hat{r}_{ij}, & r_{ij} < 1 \\ 0, & r_{ij} > 1 \end{cases} \tag{4}$$

where, γ is a friction parameter, $\omega^{D}(r_{ij})$ is a short range weight function. The random force F^{R} also acts between all pairs of beads subject to a similar short range cutoff, but with a different function $\omega^{R}(r_{ij})$ acting to pump energy into the system:

$$F_{ij}^{R} = \begin{cases} \frac{\sigma}{m_i}\omega^{R}(r_{ij})\zeta_{ij}\Delta t^{-\frac{1}{2}}\hat{r}_{ij}, & r_{ij} < 1 \\ 0, & r_{ij} > 1 \end{cases} \tag{5}$$

where, $\zeta_{ij}(t)$ is a delta-correlated stochastic variable:

$$\left\langle \zeta_{ij}(t) \right\rangle = 0 \tag{6}$$

$$\left\langle \zeta_{ij}(t)\zeta_{kl}(t') \right\rangle = (\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk})\delta(t-t'). \tag{7}$$

2.2. Mesoscale simulation parameters of PVA, Chitosan and Gentamicin

In DPD simulation, a Chitosan and a PVA monomer are represented with a single bead denoted with C and P, respectively (Fig. 1a, 1b). The polymeric chains are represented with several C and P beads. The molecular structure of Gentamicin is shown in Fig. 1c. Each part separated by dashed circles is represented with a single bead named, A, B and D, respectively.

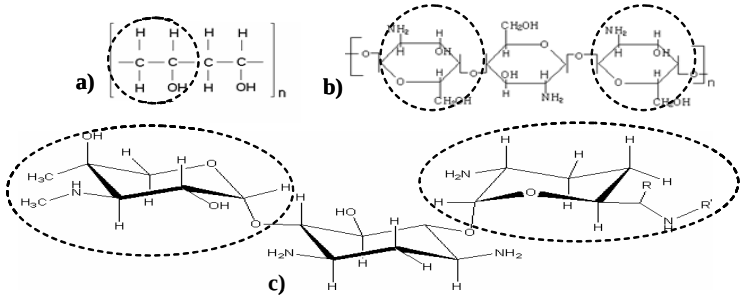

Fig. 1. DPD beads representation of: a) PVA, b) Chitosan and c) Gentamicin

The connectivity of the polymeric chain is described as C50 (consisting in 50 beads) and P70 (consisting in 70 beads), while that of the drug is A1B1D1. The connectivity of the beads was defined by calculating Flory-Huggins interaction parameters (χ). Different computational methods are available for

estimating of the χ-parameters between polymer-polymer, or polymer-drug [5-6]. In this study, χ-parameters were estimated from analysis of atomistic bulk models of single component systems. The cohesive energies were calculated for pure PVA, Chitosan and Gentamicin using molecular mechanics technique; then, solubility parameters of the components were estimated. Flory-Huggins parameter is calculated from solubility parameters using (8) [11].

$$\chi_{ij} = \frac{(\delta_i - \delta_j)^2 V}{RT} \quad \text{.....(8)}$$

where, δ_i and δ_j, are the solubility parameters of i and j, respectively, while V is the molar volume of the bead, R is universal gas constant, and T is temperature. Subsequently, Flory-Huggins parameters were converted into repulsion parameters (a_{ij}) between pairs of beads using (9) [11] and their values are listed in Table 1.

$$a_{ij} = \frac{\chi}{0.306} + 25 \quad \text{.....(9)}$$

Table 1

Repulsion parameters a_{ij} used in DPD simulations

Bead type	P	C	GA	GB	GC
P	25.00				
C	26.37	25.00			
A	27.61	36.93	25.00		
B	30.16	43.18	25.58	25.00	
D	25.62	31.02	26.17	28.66	25.00

Computational cubic bulk models of PVA-Chitosan-Gentamicin were implemented with periodic boundary conditions in all three directions in 10×10×10 r_c boxes with different compositions 0.8:0.2, 0.6:0.4, 0.5:0.5, 0.4;0.6 (w:w). The beads of the same molecule are connected by harmonic spring having the spring constant c = 4. The DPD simulations run over 100,000 steps with a time step of 0.05 reduced units (r.u.). The molecular models construction and subsequent DPD simulations are performed using the Materials Studio 4.0 software (Accelrys, Inc).

2.3. Experimental investigation of PVA - Chitosan and Gentamicin

PVA-Chitosan polymer membranes are obtained by solution casting method. Each component was first prepared as a solution: 1% w/v PVA solution was made by autoclaving for 20 minutes at 120°C in double distilled water, 1.1% w/v Chitosan solution was prepared by dissolving in 10% v/v acetic acid with constant stirring; the solution was then filtered in order to obtain a clear solution. The synthetic/natural polymer solutions are mixed under stirring in different weight ratios: 0.8:0.2, 0.6:0.4, 0.5:0.5, 0.4:0.6 (w/w), respectively. For each membrane sample, 10 mL of the blend were prepared by thoroughly stirring the

mixed polymer solutions for 5 minutes. Each blended polymeric solution was casted onto a clean dry Petri glass capsule at room temperature and dried over a period of 72 hours. For each weight ratio, dehydro thermal treatment (DHT) of 15 hours was performed, which implies the heating of the samples at 125°C under vacuum conditions for 15 hours. The homogeneity of the materials was assessed for each sample by means of Fourier transform infrared spectroscopy (FT-IR). Infrared spectra were recorded by means of FT-IR spectrometer for pure synthetic polymer, biopolymer, and for considered blends.

The cytotoxicity tests were performed using murine cell line, L-929, fibroblast, (ECACC n°85011425). After disinfection, three samples for each composition were placed in 48-well Cell Culture (plate TCPS with 48 wells) and kept in contact with 1 ml of culture medium (DMEM Sigma D6546 +L-G7513 + glutammine and antibiotic Sigma P0781) for 1, 3 and 7 days. At the end of the tests period, three plates for cell cultures with 24 wells (one for each incubation time period) has been sown with 500 µL of cell suspension containing $1.53x10^5$ cells per ml. At 1 hour after sowing, the adhesion of the cells at the bottom of wells was observed; further 1 mL of eluate was added to each well. Three wells per culture plate to which was added 1 mL of culture medium were used as control. The eluates were left in contact with the cells for 24 and 72 hours after which observation by optical microscope was performed.

3. Results and Discussions

3.1. Computational investigation of PVA-Chitosan-Gentamicin membranes

The Gentamicin distribution in the drug carrier, i.e. PVA-Chitosan membrane, was analyzed by DPD simulation. Assuming Flory-Huggins theory [11], which predicts that components with similar δ values lead to small repulsions and should mix, PVA (δ=16.84 $(J/cm^3)^{-0.5}$) should mix well with Gentamicin (δ=22.08 $(J/cm^3)^{-0.5}$) and should form uniform composites. In contrast, Chitosan (δ=13.15 $(J/cm^3)^{-0.5}$), which has a significantly different δ value, is expected not to form uniform composite with Gentamicin. At the beginning of the simulations, all the components were mixed together; after 5000 steps Gentamicin and PVA congregate, as expected according to Flory-Huggins theory. Gentamicin dispersed well in PVA polymeric matrix displaying a homogeneous distribution (Fig. 2, light grey layer). With the increase of simulation time, Chitosan polymeric chains migrated and the bulk of the material presents lamellar structure (Fig. 2). The equilibrium was reached after 20000 simulation steps. However, in order to obtain statistical results of the simulated system, in what concerns the material morphology and diffusivity, the simulation time was set to 100,000 steps. In preliminary DPD simulations using small systems size of 10×10×10 r.u. unit cell, it was confirmed that diffusivities are

affected by the blend compositions. Concerning the numerical values, diffusivity constants of the bioartificial blends predict similar diffusion constants for all the blends, with a slight decrease of the diffusion coefficients with the increase of the biopolymer (Chitosan) content.

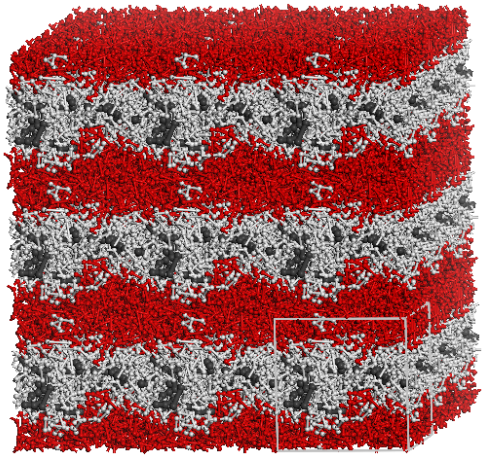

Fig. 2. Equilibrium morphology of PVA-Chitosan-Gentamicin (0.45:0.45:0.1 w/w) blends; the red chain denote a high molecular weight of Chitosan chain, the light grey chain denote a high molecular weight of PVA chain, and the intense dark grey spheres are Genatmicin molecules

The diffusion constants range from 0.13 r.u. for PVA-Chitosan 0.8:0.2 (w/w) to 0.16 r.u. for the blend PVA-Chitosan 0.4:0.6 (w/w). These results are in agreement with theoretical and experimental data from the literature. Similar trends were found by Hofmann *et al*. [1], and by Mangala *et al*. [12]. The dimension of the diffusive molecule is another parameter which influences the diffusion (connected beads in PVA chain, n = 70, n = 50 for Chitosan chain, and n = 3 for Gentamicin).

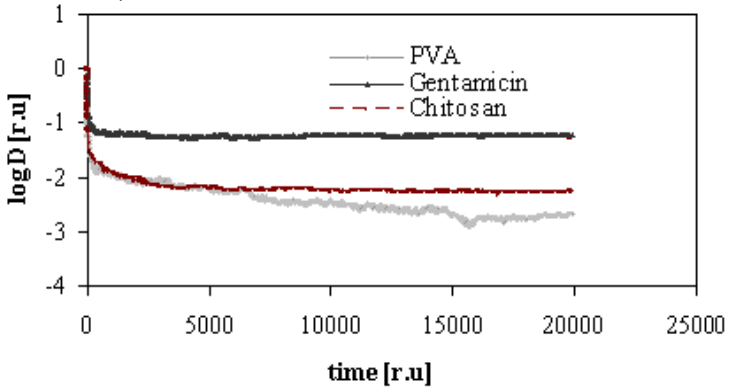

Fig. 3. Values of diffusion coefficient of PVA, Chitosan and Gentamicin

The diffusion constants decrease with the dimension of the diffusive molecule (Fig. 3). The diffusivity values of the polymer are quite large, at approximately one tenth of that for the drug particle. This trend is contrary to the actual systems described. The reason for this inconsistency might be the small number of connected particles for the polymer model (n = 70 and n = 50), but also the soft interaction of particles in the DPD system. Therefore, the lower mobility of PVA, Chitosan polymers can be attained by considering a longer polymer model in the future.

3.2. Experimental analysis of PVA-Chitosan-Gentamicin membranes

It was noticed that the PVA-Chitosan bioartificial material was stable after the DHT treatment, being clear, flexible and transparent. The homogeneity of the blends was investigated using FT-IR spectroscopy, which in the case of all samples, it shows the presence of characteristic bands in the absorbance spectrum, associated to synthetic and biological components. Fig. 4 shows the infrared spectra of Chitosan, PVA and their blend in the range of 4000-650 cm^{-1} wavelength.

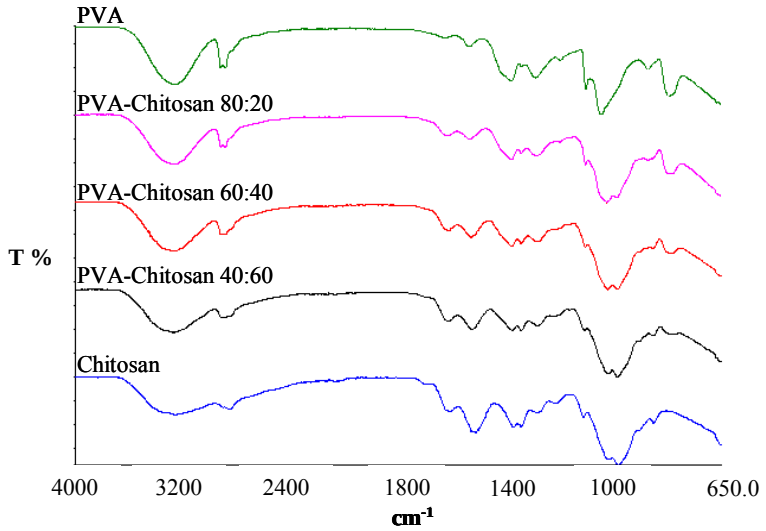

Fig. 4. FT-IR Spectra of PVA, PVA-Chitosan blend and Chitosan

In the PVA spectrum, the large peak at 3275 cm^{-1} is very evident, associated with the stretching of –OH and of –CH (2940 cm^{-1} and 2908 cm^{-1}, respectively). The bands at 1416 cm^{-1} and 1327 cm^{-1} can be associated with the bending of –OH and the wagging of –CH, respectively. The 1142 cm^{-1} event can be ascribed to PVA crystallinity and the more evident 1087 cm^{-1} peak, to the stretching of –CO. Chitosan spectrum shows the stretching of N-H (3400 cm^{-1} and

3263 cm^{-1}) and also the peak at 1545 cm^{-1} associated to the vibration of bending of N-H. PVA-Chitosan blends spectra evidenced the characteristic bands associated to synthetic and biological components. For each sample, a characteristic peak correlated with the composition of blends was observed. For example, the bands at 3275 cm^{-1}, 2940 cm^{-1} and 2908 cm^{-1} appeared to be dependent and proportional to the composition of bioartificial blends. Moreover, the peaks at 1027, 1637 cm^{-1}, characteristic only for Chitosan, decrease with the increasing of the PVA content in the blends, and these clearly demonstrate that there is intermolecular interaction between Chitosan and PVA (hydrogen bonding interaction between the functional groups of Chitosan and PVA).

Fig. 5 shows the most significant microscopy images relating to the cytotoxicity tests carried out on eluates for one day and seven days of incubation after L929 cells seeding.

Fig. 5. Optical microscopy images of L929 cell remained in contact with eluates of PVA-Chitosan 60:40 for 1day a), 7 days b) and control c)

Optical microscope images show that the attached cells to the culture plate in contact with eluates remained in contact with the PVA-Chitosan polymeric materials for 1 day (Fig 5 a), and 7 days (Fig 5 b). Fig. 5 c reports images of the control which represented L929 cells sown on TCPS. From Fig.5, one can observe strong cell vitality for all compositions of the material; furthermore the cell vitality is comparable to that characteristic for the control.

4. Conclusions

It was shown that DPD simulations combined with experimental tests are an effective methodology, providing information on phenomena that take place at mesoscale level, determining the macroscale properties of a given material. Therefore, joining molecular modeling to the experimental work would help to conduct more focused and model-oriented tests, avoiding the time-consuming trial-and-error procedure. By means of indirect cytotoxicity tests, it was demonstrated that PVA-Chitosan possess an excellent biocompatibility; therefore it is a suitable material to be used for biomedical applications. Additionally, by a simple variation of blend composition, the diffusive phenomenon can be finely tuned.

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