

Use of Optical Fiber Sensor for Monitoring the Degradation of Ac-Dex Biopolymeric Nanoparticles[†]

Marco César Prado Soares ^{1*}, Gabriel Perli ², Matheus Kauê Gomes ¹, Carolyne Brustolin Braga ², Diego Luan Bertuzzi ², Eric Fujiwara ¹ and Carlos Kenichi Suzuki ¹

¹ School of Mechanical Engineering, University of Campinas, São Paulo 13083-860, Brazil; matheuskauêgomes@gmail.com (M.K.G.); fujiwara@fem.unicamp.br (E.F.); suzuki@fem.unicamp.br (C.K.S.)

² Institute of Chemistry, University of Campinas, São Paulo 13083-970, Brazil; perligabriel@gmail.com (G.P.); carolbbraga7@gmail.com (C.B.B.); diego.bertuzzi@hotmail.com (D.L.B.)

* Correspondence: marcosoares.feq@gmail.com; Tel.: +55-19-3521-3337

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Abstract: Acetalated Dextran (Ac-Dex) is a promising pH-sensitive biocompatible and biodegradable polymer for nanomedicine applications. In this work, Ac-Dex nanoparticles were synthesized by two different solvent evaporation methods, the single nanoemulsion and the double nanoemulsion. The Ac-Dex particles were characterized by Scanning Electron Microscopy and the synthesis of highly homogeneous spherical particles was verified. Then, an optical fiber sensor based on quasi-elastic light scattering and comprised of only single-mode optical fibers and standard telecommunication devices showed sensitivity regarding the nanoparticles concentrations and was used for monitoring their degradation over 12 h under pH and temperature conditions of cancerous tissues. The results revealed a well-controlled degradation pattern, corroborating the suitability of the modified polymer to the release of active compounds in a sustainable manner and also demonstrating the applicability of the sensor for the *in-situ* evaluation of the degradation.

Keywords: optical fiber sensor; Ac-Dex nanoparticles; concentration and degradation monitoring.

1. Introduction

Current research in the nanomedicine field is focused on the development and application of new biocompatible and biodegradable materials. Nanostructures produced from degradable polymers such as polysaccharides have been investigated as carriers for pharmaceuticals [1]. The main importance of these biomaterials for drug delivery is related to their tunability for releasing active compounds in response to pH changes. Thus, the progressive and selective delivery of the drugs in mildly acidic diseased tissues (pH 5.0-5.5) minimize their adverse effects on healthy cells and increase their therapeutic efficiency [2].

Acetalated Dextran (Ac-Dex) is a promising example of pH-responsive polymer. Its precursor, the water-soluble dextran, is a bacterially derived homopolysaccharide of glucose, FDA-approved, and widely available [3,4]. In turn, Ac-Dex is a hydrophobic material prepared by masking the hydroxyl groups of dextran with acetal groups and is easily processable by different emulsion techniques [2,5]. Ac-Dex nanoparticles (NPs) present the ability to encapsulate both hydrophobic and hydrophilic molecules, depending only on the emulsion process employed during their synthesis. Particles prepared by oil-in-water (o/w) or water-in-oil-in-water (w/o/w) emulsions are able to encapsulate either hydrophobic or hydrophilic molecules, respectively [5].

In this work, colloidal dispersions of Ac-Dex were synthesized by two different routes, the single-emulsion (o/w) and the double-emulsion (w/o/w), referred as Ac-Dex SE and Ac-Dex DE, respectively. Scanning Electron Microscopy (SEM) was employed to investigate the morphology of Ac-Dex nanoparticles synthesized through these two methodologies and an optical fiber sensor [6,7] was employed to assess the colloidal behavior of Ac-Dex NPs over time. More specifically, the stability of a colloidal set was investigated during 12 h in an acidic environment (pH 5.5) under 37°C, which corresponds to the cancer tissue conditions.

2. Materials and Methods

2.1. Polymer Synthesis and Nanoparticles Preparation

The Ac-Dex polymer was synthesized using a Schlenk under atmosphere of nitrogen. Dextran (M_w 10,500 g/mol, 1.00 g, 0.095 mmol) was dissolved in anhydrous DMSO (20 mL) and the catalyst pyridinium *p*-toluenesulfonate (15.6 mg, 0.062 mmol) was added. Then, 2-methoxypropene (3.4 mL, 37 mmol) was injected using a disposable syringe and a needle. The reaction was quenched with triethylamine (1 mL, 7 mmol) after exactly 60 min. The product was precipitated in ice-cold dd-H₂O (500 mL, pH 8) and filtered through Celite. Chloroform was added to solubilize the solid, followed by the removal of the organic solvent, affording the Ac-Dex polymer as a white crystalline powder. Then, polysaccharide-based nanoparticles were prepared by using single- and double-emulsion processes [5] (Figure 1). In both methods, it was employed 5 mL of poly(vinyl alcohol) (PVA; M_w 13,000-23,000 g/mol, 87-89% hydrolyzed) solution (3% wt/wt in PBS) and a probe sonicator (Digital Sonifier S-450, Branson Ultrasonics, Danbury, CT, USA) with a duty cycle of 50% of amplitude for 60 s.

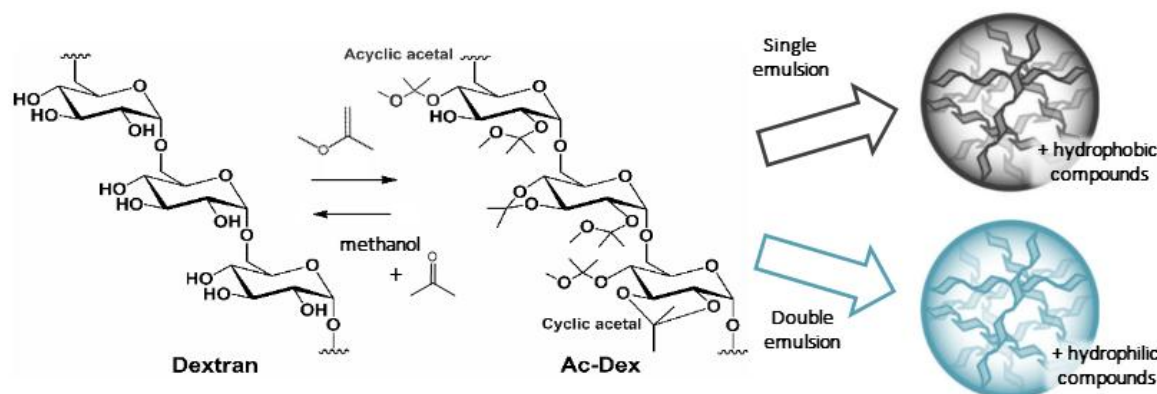


Figure 1. Synthesis/chemical degradation of Ac-Dex polymer and nanoemulsion process.

The dextran hydroxyl groups were protected with acetals by using 2-methoxypropene in the presence of acid catalyst. Depending on the reaction time and temperature, different proportions between cyclic and acyclic acetals are obtained through this reaction [9]. The acetal groups make the new polymer hydrophobic and pH-sensitive, which provides a trigger for acid-mediated degradation. It is well-known that the acetal groups suffer hydrolysis when submitted to weakly acidic pH conditions (pH=5.5), what unmasks the parent hydroxyl groups of dextran and converts the hydrophobic Ac-Dex to its hydrophilic precursor (dextran), also forming small amounts of acetone and methanol (Figure 1). On the other hand, degradation by hydrolysis at neutral pHs is minimal, precluding non-controlled released in the bloodstream and providing a higher release rate in diseased tissues [5].

2.2. Nanoparticles Characterization

The morphological investigation of the nanostructures was carried out using the field emission scanning electron microscopy (FESEM: Quanta 250 FEG, Thermo Fisher Scientific, Waltham, MA, USA), operated at a voltage of 5 kV. The Ac-Dex nanoparticles were suspended in phosphate buffer

solution (pH 7.4) and dripped onto silicon wafers. The samples were dried overnight under a hood and sputter-coated with a 2 nm layer of metallic silver.

Representative figures of the SEM results obtained for Ac-Dex SE and Ac-Dex and DE nanoparticles were chosen for the diameter analysis with a MATLAB (MathWorks) image processing routine that identifies the particles' centroids, counts the centroids, and evaluates each diameter. The mean diameter, the uncertainty of the real measurement (95% confidence interval of the mean value) and the corresponding histograms were then obtained by counting 89 Ac-Dex SE and 1398 Ac-Dex DE NPs. The confidence intervals were calculated using the One-Way ANOVA test of Minitab 17 Statistical Software (Minitab Inc).

2.3. Optical Fiber Sensor for in-situ Assessment of Degradation

The optical fiber sensor used for the analysis of degradation over time is comprised of standard telecommunication devices: light is emitted by a laser diode (CW, 1310 nm) and launched into the silica single-mode waveguide. The fiber end-face, which is plane-polished and enclosed in zirconia ferrule, works as the sensing probe and is immersed in the analyzed sample. Part of the light is reflected at the silica-liquid interface due to the refractive indexes difference (modulated according to Fresnel equations) and subsequently delivered to a photodiode by means of a coupler. The sensor is calibrated for collecting 20,000 intensity measurements at 1 kHz rate. Finally, the acquired signals are processed by routines developed in MATLAB [6].

In colloidal dispersions where the particles dimensions are comparable to the laser wavelength, the interaction of the light with the particles produce quasi-elastic light scattering (QELS). As the scattered rays are coupled to the fiber, temporal variations emerge in the reflected intensity signal $I_R(t)$. Given $G_2(\tau)$ the autocorrelation of I_R as a function of a delay time τ , the colloidal properties can be estimated from the Siegert relation [10], Equation 1.

$$G_2(\tau) = A + B \cdot \exp(-2\Gamma_m \tau) \quad (1)$$

In Equation 1, A is the baseline, B is the coherence factor, and Γ_m is the average decay rate, which is function of the magnitude of the scattering vector q and the particle diffusivity D_{AB} (Equation 2). According to the Stokes-Einstein diffusivity model, D_{AB} is inversely proportional to the particles' average diameter, and then Γ_m increases as the particles' dimensions decrease [10,11].

$$\Gamma_m = D_{AB} q^2 \quad (2)$$

Firstly, calibration curves are obtained for inferring the concentration of Ac-Dex SE and Ac-Dex DE particles dispersed in acetate buffer (pH 5.5) with concentrations ranging from 0.3 to 10 mg/mL. The full setup presenting the electronics, the analysis of an Ac-Dex suspension and the scattering of the light is shown in Figure 2. Then, a suspension of Ac-Dex DE (initial concentration of 4 mg/mL) was continuously analyzed over 12 h for evaluating its degradation under mildly acidic pH (5.5 buffer): as the colloidal particles are disassembled, the decrease of Γ_m value is expected [6]. All the colloidal suspensions used in the experiments were kept in an ultrasound temperature-controlled bath (5.9L Ultrasound Bath, Ultronique Eco-Sonics, Brazil) under 37°C for simulating conditions similar to tumoral tissues [8].

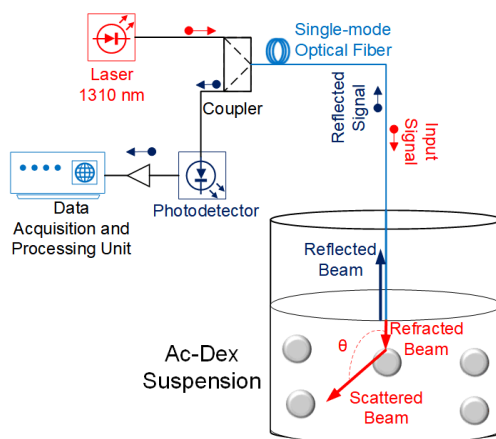


Figure 2. Full experimental setup, representing the electronics and QELS.

3. Results and Discussion

3.1. Nanoparticles Characterization

The SEM images for Ac-Dex NPs synthesized by single- and double emulsion are shown in Figures 3A and 3B, respectively. The Ac-Dex NPs present almost perfectly smooth surfaces, spherical shapes and low polydispersity (Figure 3).

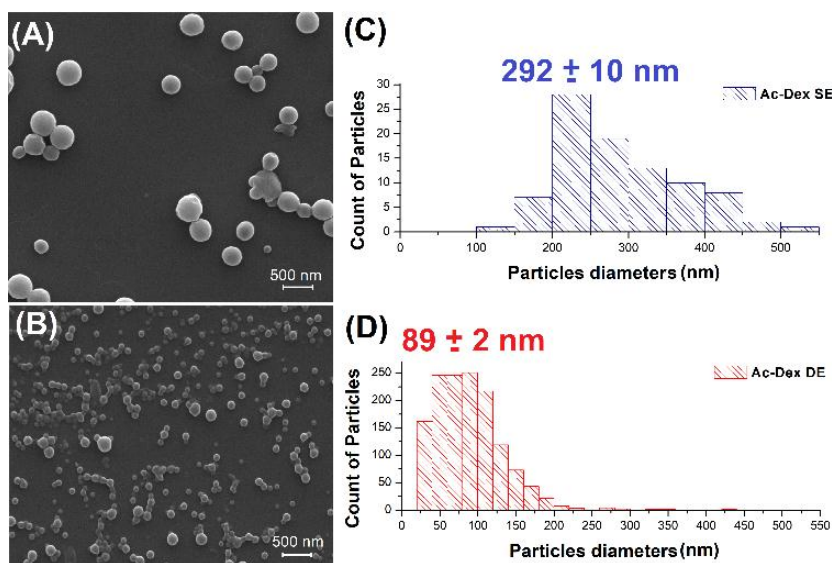


Figure 3. SEM image obtained for (A) Ac-Dex SE NPs, and (B) Ac-Dex DE NPs; histograms and mean diameters correspondent to (C) Ac-Dex SE NPs, and (D) Ac-Dex DE NPs.

3.2. Optical Fiber Sensor Dynamic Evaluation

The reflected intensity signal and the autocorrelation function obtained for the solution of Ac-Dex SE in the concentration of 10 mg/mL are shown in Figures 4(A) and 4(B), respectively. It can be noticed the high dispersion of the data, that is characteristic from the QELS phenomenon, and the exponential-shape of the autocorrelation function $G_2(\tau)$, in agreement to Equation 1. The Γ_m values obtained for each tested sample are, in turn, summarized in Figure 4(C). As expected, the higher concentrations indeed resulted in higher Γ_m due to the increase on the number of particles able to scatter the light, and the lower average diameter resulted in a higher rate for a given concentration, as a consequence of the increase of the diffusivity [10,11].

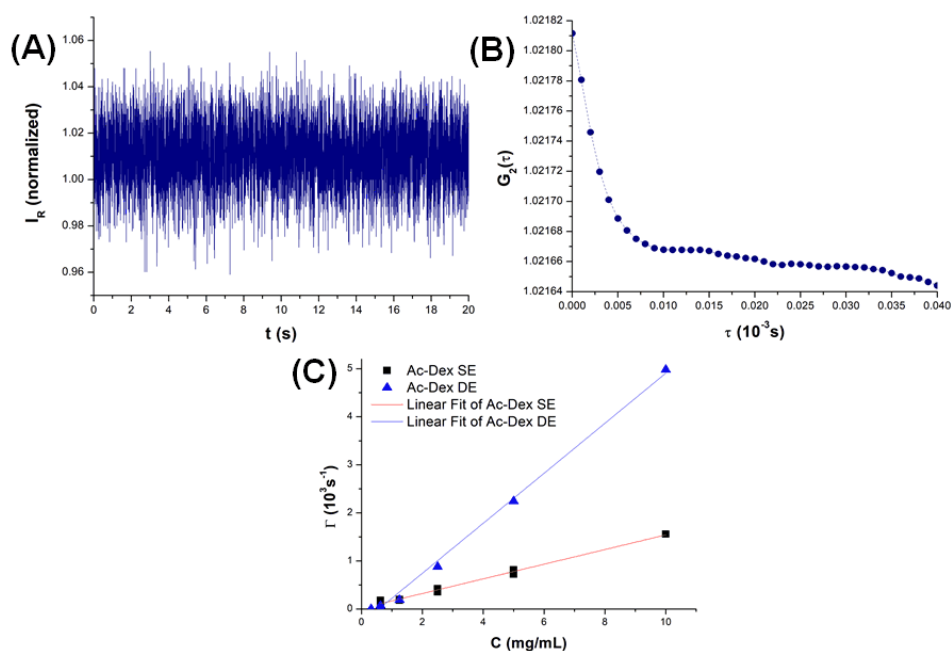


Figure 4. (A) Reflected intensity I_R ; and (B) $G_2(\tau)$ obtained for 10 mg of Ac-Dex SE /mL; (C) Mean decay rates Γ_m obtained in the optical fiber sensor and correspondent linear fits.

The angular coefficient of the equation obtained for Ac-Dex DE sample is significantly higher compared to the result for the Ac-DEX SE NPs, which is in agreement with the theory of dynamic light scattering since smaller particles diffuse faster. The linear regressions resulted in the calibration functions $\Gamma_m = 0.152C + 0.017$ (Pearson correlation coefficient $R^2 = 0.9915$) and $\Gamma_m = 0.520C - 0.295$ ($R^2 = 0.9952$) for Ac-Dex SE and Ac-Dex DE, respectively. In both equations, Γ_m is expressed in 10^3 s^{-1} and the nanoparticles concentration C is in mg mL^{-1} . The sensitivity of the sensor is simply given by the ratio between the signal variation to the concentration variation, *i.e.*, by the angular coefficient of each linear regression: $0.152 \times 10^3 \text{ s}^{-1}\text{mg}^{-1}\text{mL}$ for Ac-Dex SE and $0.520 \times 10^3 \text{ s}^{-1}\text{mg}^{-1}\text{mL}$ for Ac-Dex DE. Due to the higher sensitivity, the Ac-Dex DE particles (suspension with initial concentration of 4 mg/mL) were chosen for the degradation test (Figure 5).

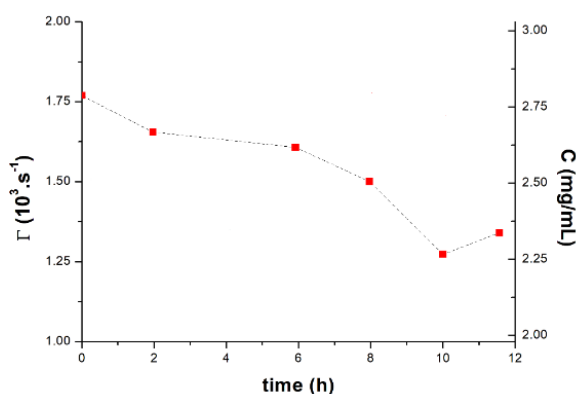


Figure 1. 12-h degradation test for Ac-Dex DE. The main vertical axis represents the decay rate obtained by the sensor and the secondary axis shows the calculated concentration.

It is possible to verify that the particles degrade in the presence of the acid pH characteristic of the cancer tissues. However, this process does not occur immediately, but in a tunable way (degradation of 20.9% of the NPs after 12 h). This result suggests that Ac-Dex has great potential for applications in which it is necessary to provide a progressive release of medication, maintaining a minimum therapeutic concentration on the tissue throughout the treatment.

4. Conclusions

The successful synthesis of Ac-Dex nanoparticles by two different methods was reported, resulting in statistically significant differences on the average diameter (292 and 89 nm for Ac-Dex SE and Ac-Dex DE, respectively). Each particles dispersion was analyzed with the optical fiber sensor, leading to distinct sensitivities (0.152×10^3 and $0.520 \times 10^3 \text{ s}^{-1}\text{mg}^{-1}\text{mL}$ for SE and DE, respectively) as a consequence of the different diffusivities. The continuous evaluation of Ac-Dex DE with the sensor showed that this nanomaterial is adequate for drug-delivery, since it suffers a controlled degradation under conditions that simulate those in tumoral tissues, whereas the optical fiber sensor showed itself a feasible instrument for the *in-situ* monitoring of the colloidal system. Future works will focus on chemically modifying the optical fiber with targeting groups to co-evaluate the colloidal properties while the release of active compounds under tumoral acidic conditions is quantified.

Author Contributions: M.C.P.S wrote the paper and performed the optical fiber sensor experiments and data analysis, G.P. prepared the nanomaterials, the characterization and supported the paper writing, M.K.G. designed the computer routine for image processing and analyzed the SEM images, C.B.B. and D.L.B. gave support on the nanomaterials preparation and characterization E.F. and C.K.S. gave funding and instrumentation support, project coordination and reviewed the paper.

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