



Systematic Study of NaF Nanoparticles in Micelles loaded on Polylactic Acid Nanoscaffolds: In Vitro Efficient Delivery

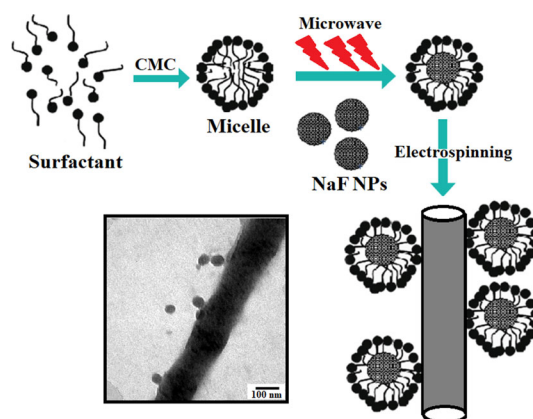
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Abstract

The overall aim of this work was developing and optimized NaF nanoparticles in micelles structures loaded on polylactic acid nanoscaffolds as biocompatible and biodegradable polymers for use in dental delivery. In this research, NaF nanoparticles were successfully synthesized in micelles structures and loaded on the biopolymers on as dental resins through direct micelle assisted electrospinning method. NaF nanoparticles as a heterogeneous structure were dispersed in biopolymers nanoscaffolds which makes it suitable in dental delivery. The synthesis parameters for preparation of NaF nanoparticles loaded on polylactic acid nanoscaffolds were evaluated by design of experiments and the process control was systematically studied using analysis of variance. Products were characterized with scanning electron microscopy, transmission electron microscopy, atomic force microscopy, Fourier-transform infrared spectroscopy, thermogravimetric analysis, Brunauer–Emmett–Teller and nitrogen adsorption isotherms. In vitro drug release of NaF nanoparticles loaded on polylactic acid nanoscaffolds was studied with Franz Cells and data release was investigated through UV–vis spectroscopy about $\lambda = 486$. The obtained results showed the released value of NaF nanoparticles from the formulation containing 0.5% PLA at $\lambda = 486$ nm about 84% after 240 min.

Graphic Abstract



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Introduction

In fact, fluoride inside the body naturally occurs in the form of calcium fluoride [1, 2]. This material can be found more often in bones and teeth [3]. Effect of fluoride on dental tissues during and after exposure to minerals may affect enamel [4], cement or dentin [5], and the presence of sufficient fluoride increases the amount of fluorapatite compared to hydroxyapatite during tooth extraction [6]. Lately, the encapsulation of lipophilic and hydrophilic drugs in nanostructured systems has created a new and innovative way to increase the encapsulation efficiency (EE %) and drug delivery [7], hence nanocarriers especially nanoscaffolds with high efficiency have developed solubility of conventional drug delivery systems [8] some of the biodegradable and biocompatible polymers such as polylactic acid recently they have been widely used in various medical fields [9].

Nanotechnology as a new viewpoint offers great ability to control and the release of hydrophobic and hydrophilic drugs [10]. Materials for dental restorations such as fluoride, as a naturally-occurring mineral, helps prevent cavities in teeth also protect against recurrent caries following a restoration placement [11]. The nanoparticles systems due to high surface to volume ratio attracted the attention of a large number of researchers [12] and suggested as high powerful tools for improving the efficacy for dental delivery, the physical size of the nanoparticles plays a key and an important role in oral cavity retention [13]. There are numerous investigations on the synthesis routes of nanomaterials crystal structure have been carried out, many of these approaches such as solid state [14], hydrothermal [15], solvothermal method [16, 17], citrate complex route [18], require heat-treating or high pressure for several hours [19]. These problems can be resolved by applying DMAE method. The direct micelle method as a good, simple, convenient synthesis, the cost-effective synthetic

procedure can be used for the synthesis of the uniform crystal-nano structure composites and nanocrystals [20]. Non-ionic surfactants have more chemical stability against both oxidation and temperature with high potential such as sustained release action appropriate these structures to many pharmacological agents for their action against various diseases [21–24]. The effect of different synthetic conditions such as reaction time, concentration of precursors, pH of the solution, different surfactants, sound wave intensity, and stirring on the morphologies and size of the final products were investigated with by Taguchi technique. This present work for the first time reports synthesizes and modified NaF nanoparticles loaded on polylactic acid nanoscaffolds by a chemical process for the investigation of efficient encapsulation and efficient delivery properties also new nanomaterials as a dental resins drug. In the recent years, there is a lot of interest in researches about crystal-nanostructure composites Table 1. PLA nanoscaffolds as an environmentally friendly and comfortable polymer can be used as suitable substrates and novel fillers for dental composites. The final products were characterized with X-ray diffraction (XRD), scanning electron microscopy (SEM), transition electron microscopy (TEM), Fourier transformed infrared spectrum (FT-IR), thermo-gravimetric analysis (TGA) and atomic force microscopy (AFM). Statistical data showed that factors such as pH amount, NaF concentration, microwave power and interaction between these factors, considerably affected the properties of the NaF crystal nanostructures loaded on polylactic acid nanoscaffolds formulation. It could be seen that the polymer matrix improves the mechanical properties and distribution of NaF nanoparticles. Also, the results indicated that PLA films with NaF crystal-nano structures could be considered a potential environmental-friendly as dental resins material.

Methods and Experimental

Materials and Physical Measurements

NaF powder was purchased from Chengxin Chemical Co., Ltd (Taizhou, China), sodium dodecyl sulfate (SDS) pure

Table 1 Some of researches for nano emulsion based nanoparticles in recent years

Types of nano emulsion	Drug in nano emulsion	Synthesis method	Size of nano emulsion	References
CaO nanoparticles	Fluoride removal	Sol–gel	50 nm	[26]
Protein complex	Saffron	Coprecipitation	< 100 nm	[27]
Ti-doped hematite	NaF	Hydrothermal synthesis	340 nm	[28]
Nanoemulsion formulation	Sefsol 218, Carbitol	Thermodynamically	80.9 nm	[29]
Tween-80 as emulsifiers	Curcumin	Nanoemulsion	141.6 ± 15.4 nm	[30]

99% and NaOH analytical grade without any purification were purchased from Merck Co. The micelles in this study were synthesized through co-precipitation method. Polylactic (PLA) granule, 3 mm nominal granule size powder with purity > 99% were purchased from Sigma-Aldrich. Electrospinning set up with a high-voltage-power supply capable generating voltages up to 30 kV, was used as the source of the electric field to preparation of nanofibers. The surface morphology of NaF nanoparticles loaded on polylactic acid nanoscaffolds was examined by scanning electron microscopy (SEM) LEO 1455VP Razi Metallurgical Research Center (RMRC). Dynamic light scattering (DLS) was used to study the NC hydrodynamic sizes. The DLS experiments were performed using laser-light scattering Mastersizer 2000E, Malvern Instruments, UK. Transmission electron microscopy (TEM) images were obtained on a Philips EM208 transmission electron microscope with an accelerating voltage of 200 kV in University of Shahid bahonar Kerman-IRAN. Thermogravimetric analysis (TGA) were calculated with the gravimeter S-15000 model under a nitrogen with the heating rate of 10 °C/min from 100 °C up to 400 °C. Fourier transform infrared (FT-IR) spectra were recorded on Shimadzu Varian 4300 spectrophotometer in KBr pellets. Atomic force microscopy (AFM) image has been calculated with Nano wizard II JPK Made in German. UV–visible spectrum of product was recorded using Scinco UV–vis scanning spectrometer (ModelS-4100). The N₂ adsorption–desorption isotherms of nanoemulsion gel were recorded by using Belsorp II-BEL equipment (BEL Japan Inc., Osaka, Japan). Analytical HPTLC was performed using the HPTLC system (CAMAG, Muttenz, Switzerland) that was controlled by win CATS Software.

Preparation of NaF Crystal Nanostructures

At the first for the preparation of NaF crystal nanostructures, different concentrations of NaF were dispersed in distilled water as the aqueous phase. Emulsifiers such as fatty acid esters of sorbitans (Span) and polyoxyethylene sorbitans (Tween) were employed for use in parenteral emulsion drug delivery. Researches have shown that the oil phase could aid in increasing the drug encapsulation rate due to the optimization of drug solubility within the inner oil phase. The microemulsions were spontaneously manufactured undergoing two steps, at the first oil phase and surfactants were mixing with the assistance of external energy in room temperature. The pH of the microemulsions solution has been adjusted to 7.5–8.3 using 8 ml NaOH 1 M solution dropwise to the microemulsion solution under vigorous stirring with 300 rpm at 50 °C after thermal treatment the

The mixtures were loaded into a microwave Teflon container, and then the reactions were performed in a microwave digestion system under various conditions. In the final step the solutions were allowed to cool at room temperature.

Preparation of NaF Crystal Nanostructures in Micelles

In this step at the first specified values of the sodium dodecyl sulfate (SDS) as surfactants agent, with different percentage ratio were dissolved in 10 ml of distilled water in a round bottomed flask. The solvent was evaporated to dryness in a rotary evaporator under reduced pressure at room temperature. During drying, the flask was rotated at 100 rpm speed until a smooth dry milky solution was obtained. Finally, a stable solution was formed at the critical point. The milky solution formed was hydrated using double distilled water. Then in a new method 30 mg of NaF crystal nanostructures solved in 10 ml ethylene glycol then in the next step, the above solution was added to the made non-ionic surfactant-based vesicle as in situ under reflux system at 50 °C for 120 min. A clear milky suspension was loaded into a microwave Teflon container, and the reactions were performed in a microwave system under various irradiation and time conditions. After microwave irradiation the system was allowed to cool to room temperature naturally then final products were characterized and photoluminescence properties studied.

Fabrication of PLA/NaF Nanofibrous Composites

The solutions 1 to 2 ratio PLA:NaF crystal nanostructures in non-ionic surfactant-based vesicle were supplied through 5 ml plastic syringe attached to a tip with an inner diameter 0.4 mm and the feed rate of the solution was controlled at 1.0 ml/h. The pieces of aluminum sheets were attached to a rotating metal drum (diameter of 6 cm) which functioned as the fiber collector and the grounding electrode. A fixed electrical potential of 15 kV was applied across the distance of 10 cm between the tip of the nozzle and the outer

Table 2 The factors and their levels considered for the experimentation

Process parameters	Levels	
	Level 1	Level 2
pH	7.5	8.3
Power	600	900
Cons NaF	0.05	0.1

surface of the drum. The rotational speed of the drum was 100 rpm. The electrospun time used was 2 h and all of the electrospinning procedures were carried out at 25 °C. After doing the tests, the system was allowed to cool to room temperature naturally, and the obtained precipitations were collected. In finally the products were characterized by SEM, TEM, XRD, AFM, and EDAX.

Results and Discussion

Experimental Design

Taguchi method presents a new design of experiments for the parameters optimization, the experimental design proposed by Taguchi involves using orthogonal arrays to organize the parameters affecting the process and the levels at which they should be varied. According to the Taguchi method, if three parameters and 2 levels with a one center point for each parameters L6 orthogonal array should be employed for the experimentation. Table 2 shows the superplastic forming factors and their levels considered for the experimentation. Overall seven experiments were conducted and S/N ratios value was calculated from the equation below (Eq. 1):

$$S/N = -10 \log(\text{MSD}) \quad (1)$$

Mean squared displacement (MSD) is a measure of the deviation of the position of a particle with respect to a reference position over time. Confirmation tests were conducted using the higher the better values for dome height and thinning factor. The influence of parameters was found by Taguchi technique. Figure 1a–d shows normal probability plot, versus fits, histogram and versus order, respectively for the effects of pH conditions, microwave power and concentrations of NaF on the size and particle size distribution of products. The results show that All of three parameters and interaction between them have a direct effect on the particle size of final products.

Structural Characterization

To characterize phases and examination of the crystallite size of the NaF nanoparticles X-ray diffraction pattern analysis was served. The mean diameter and distribution particle size of the crystallites can be calculated from the XRD peaks and Sherrer equation (Eq. 2).

$$D = k\lambda/\beta \cos \theta \quad (2)$$

where D is mean crystallites size, λ is X-ray wavelength (1.54056 Å), β is broadening of the line measured at half its maximum intensity (in radius), θ is diffraction angle from Bragg planes and k is shape factor (0.9). based on the

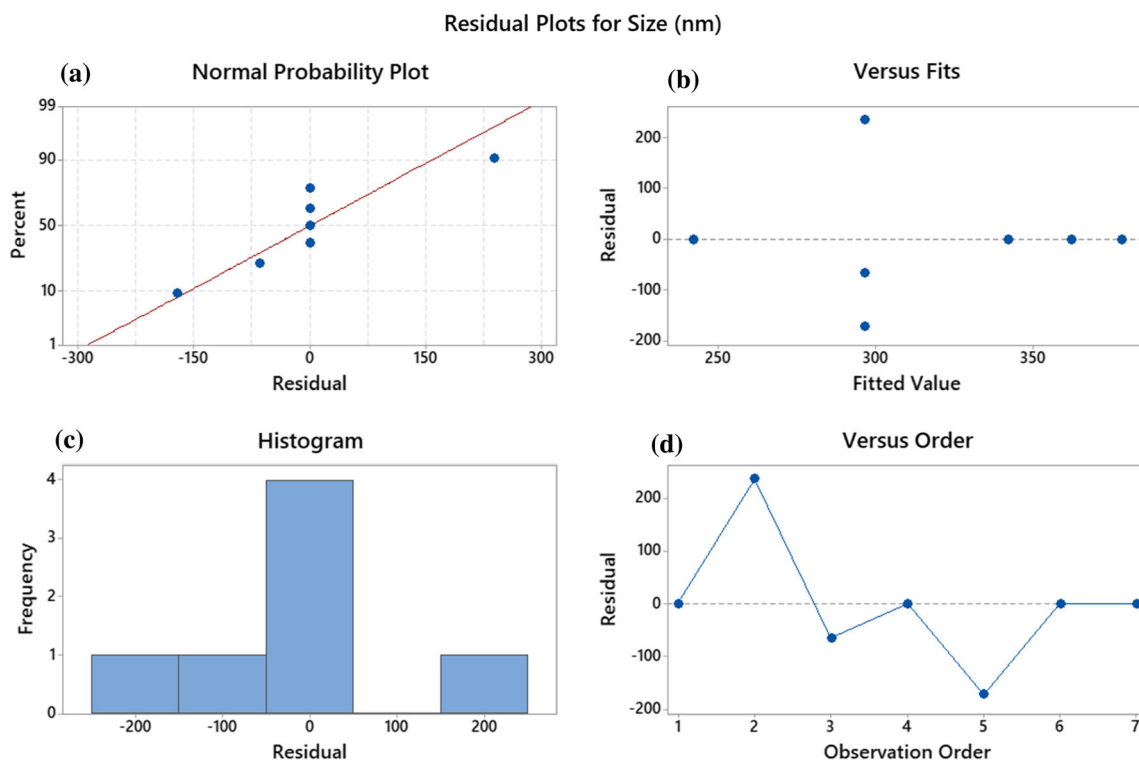


Fig. 1 Normal probability plot (a), versus fits (b), histogram (c) and versus order (d) for the effects of pH conditions, microwave power and concentrations of NaF on the size and particle size distribution

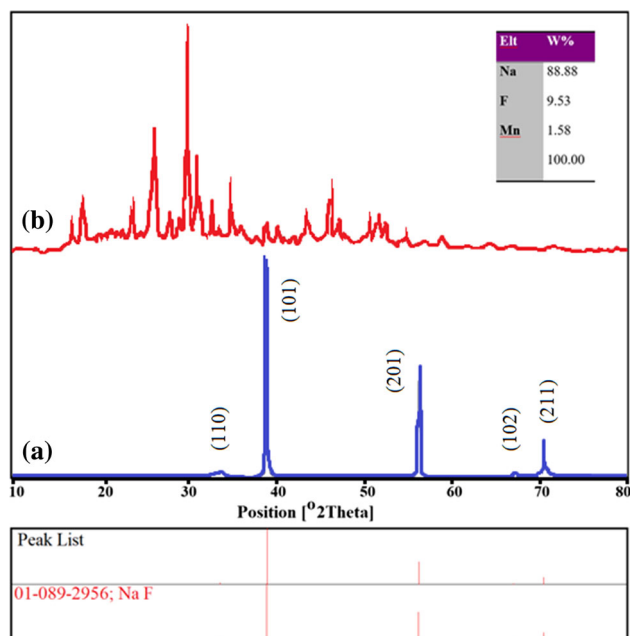


Fig. 2 XRD patterns of the NaF nanoparticles (a) and NaF nanoparticles loaded on poly(lactic acid) nanoscaffolds (b)

Using Bragg's rule, the lattice parameter was obtained from the crystalline face space (d) with the help of the following lattice parameter formula (Eq. 3).

$$a = d(h^2 + k^2 + l^2)^{1/2} \quad (3)$$

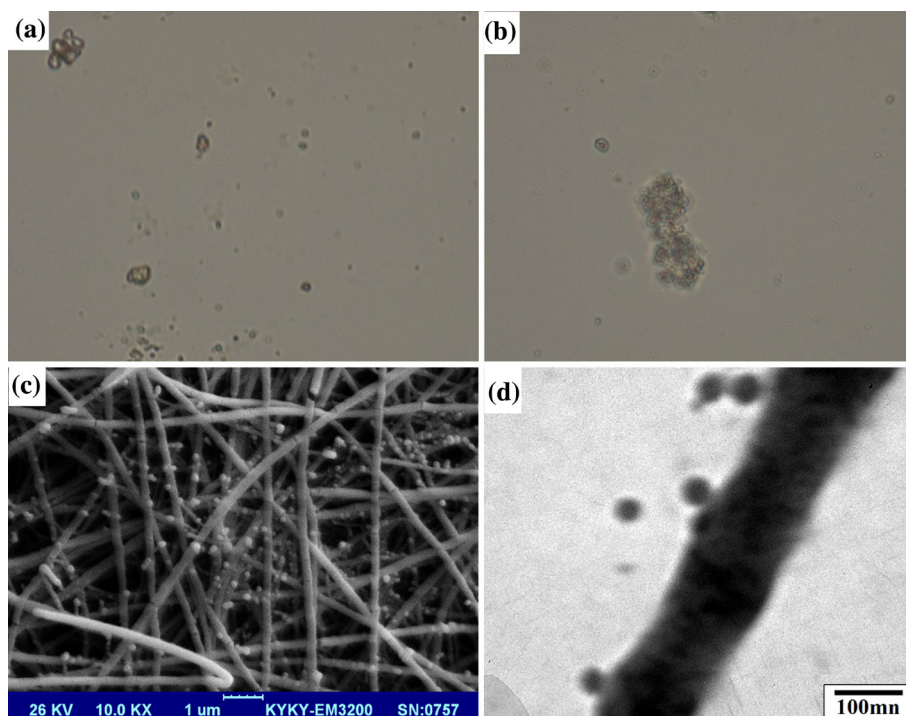
where h , k , l are the crystalline face indexes. Figure 2a and b show the XRD patterns of NaF nanoparticles and NaF

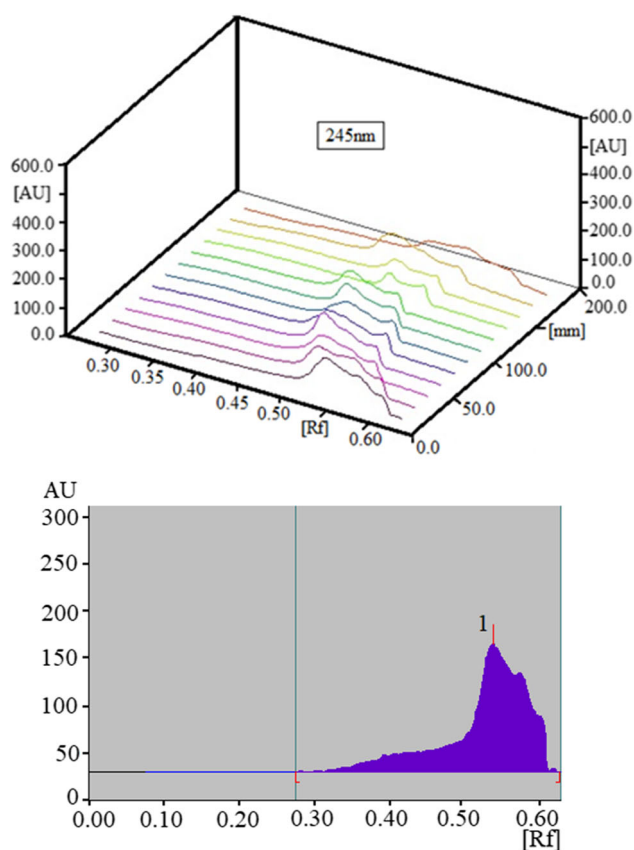
nanoparticles loaded on poly(lactic acid) nanoscaffolds, respectively. The results indicated that nanoparticles and final products were well crystallized and the characteristic diffraction peaks of the NaF nanoparticles were easily indexed to the monoclinic structure of NaF nanoparticles at $2\theta = 33.65, 39.02, 56.25, 67.07$ and 70.46 with Miller indices (110), (101), (201), (102) and (211) which indicated cubic (JCPDS NO. 01-089-2956).

The morphological and structural characteristics of the synthesized micelles were observed with micrographs ($450\times$ magnification). The images in Fig. 3a, b are well illustrated the formation of multilayer neosomal spaces at room temperature.

It can be clearly observed that a large number of non-ionic surfactant-based vesicles were formed with the distinct difference in distribution on a cross-section. The morphology, particle size and surface chemical properties of the NaF nanoparticles loaded on poly(lactic acid) nanoscaffolds were observed with SEM analysis. According to Fig. 3c, NaF nanospheres with regular morphology and particle size about 100 nm, as separate nanostructures placed on a polymer film. In order to demonstrate the clarity of nanoparticles and further detailed structural information of the NaF nanospheres on polymer structures, the transmission electron microscopic technique (TEM) photographs of the products are given in Fig. 3d. The size of the nanoparticles estimated from the TEM images is in close agreement with the SEM studies which shows a size of about 80–300 nm.

Fig. 3 Micrographs ($\times 450$ magnification) multilayer neosomal spaces at room temperature (a, b), SEM (c) and TEM (d) image of NaF nanoparticles loaded on poly(lactic acid) nanoscaffolds





135.3 AU	135.3 AU	135.3 AU	135.3 AU	135.3 AU	135.3 AU
0.54 Rf	0.54 Rf	0.54 Rf	0.54 Rf	0.54 Rf	0.54 Rf
0.47 Rf	0.47 Rf	0.47 Rf	0.47 Rf	0.47 Rf	0.47 Rf
0.6 AU	0.6 AU	0.6 AU	0.6 AU	0.6 AU	0.6 AU

Fig. 4 HPTLC analytical system of NaF nanoparticles loaded on poly(lactic acid) nanoscaffolds

HPTLC analytical system of NaF nanoparticles loaded on poly(lactic acid) nanoscaffolds as an entire concept that includes a widely standardized methodology were displayed in Fig. 4. Based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis HPTLC of products is able to generate a chromatographic fingerprint in the form of a unique sequence of peaks corresponding to the analyzed sample in its fullness. The aim of this analysis was obtained resulting fingerprints of products that can be compared with respect to the number, sequence, position (Rf) and colour of the separated zones. For this purpose, about 30 μ l of the NaF nanoparticles in micelles loaded on poly(lactic acid) nanoscaffolds dissolved in ethanol were automatically injected to a 20 $\times$ 10 cm silica gel HPTLC plate (Merck, Darmstadt, Germany) containing green fluorescent F254 the plates

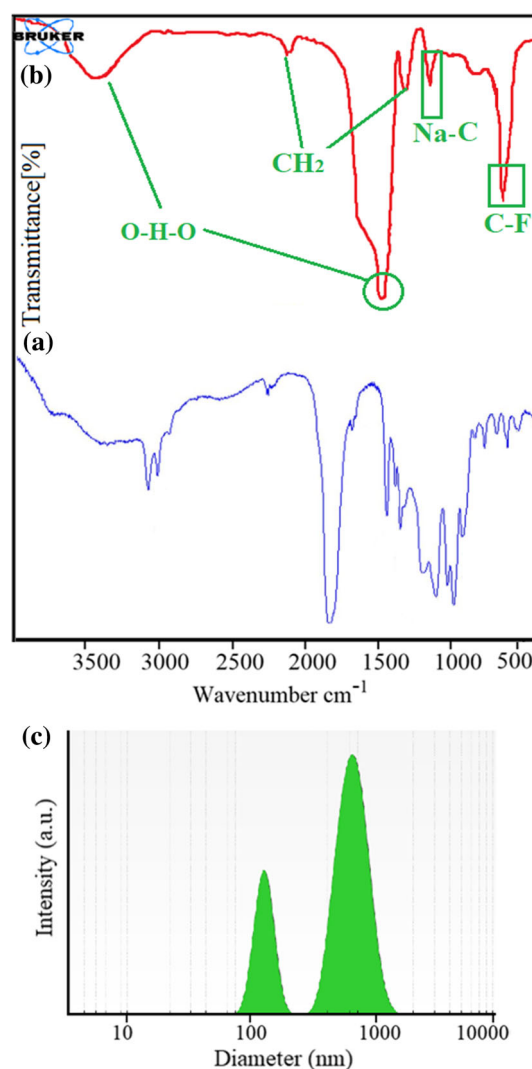
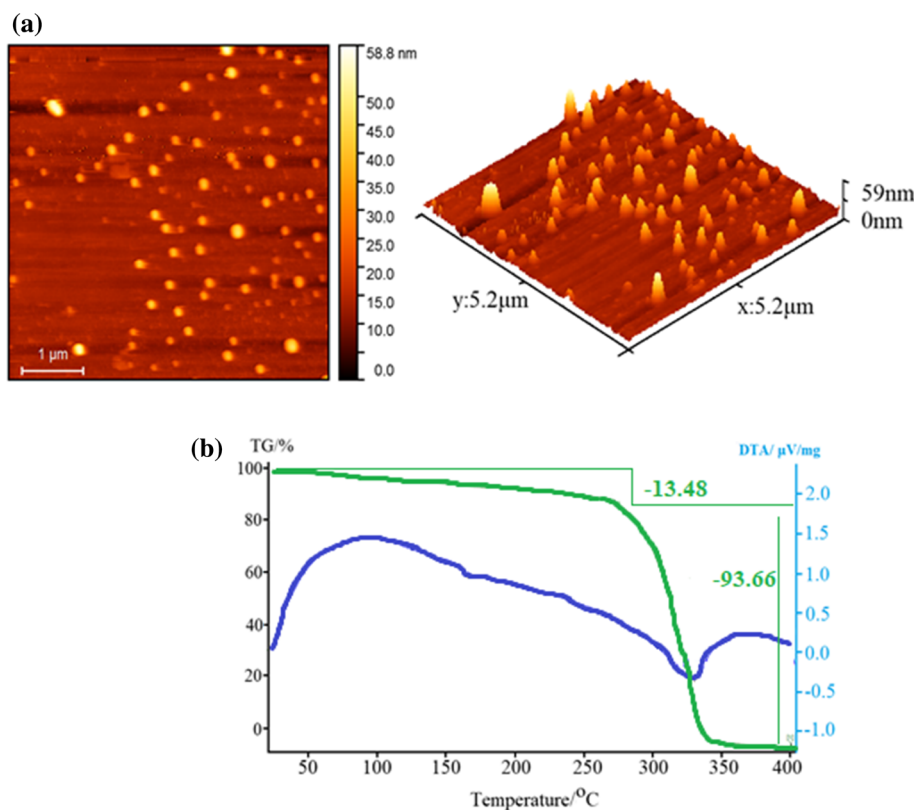


Fig. 5 The FT-IR spectra of poly(lactic acid) (a), as-synthesized NaF nanoparticles loaded on poly(lactic acid) nanoscaffolds (b) and DLS diagram of the final products (c)

were then developed with solvent mixture containing chloroform:methanol:water (65:25:4, v/v/v) up to migration distance of 60 nm. The UV-visible spectra of the NaF nanoparticles in micelles loaded on poly(lactic acid) nanoscaffolds (final concentration of 0.5 mg/ml) were recorded in the range of 200 to 600 nm using a UV-Visible spectrophotometer (PerkinElmer, Waltham, MA, USA).

Fourier transforms infrared (FTIR) spectroscopy as an experimental technique can be used initially for qualitative and quantitative analysis of organic compounds, providing specific information on molecular structure, chemical bonding and molecular environment. Figure 5a, b show the FT-IR spectra of poly(lactic acid) and as-synthesized NaF nanoparticles loaded on poly(lactic acid) nanoscaffolds. In this spectrum, two strong IR absorption bands within about $\sim$ 3400/cm and 1550/cm are due to the stretching

Fig. 6 AFM topography images (a) and TGA diagram (b) of the NaF nanoparticles loaded on polylactic acid nanoscaffolds



vibration of OH groups of H–O–H bending as for the H₂O molecules [25]. In fact, due to the high surface to volume ratio of the nanoparticles, they can absorb more water molecules at their surfaces than the bulk ones, and a weak peaks centered at 1100/cm and ~ 850/cm can be attributed to the bending vibration of the C–Na and C–F band in [C...Na...F...C] structures, respectively. The band at ~ 2450/cm and ~ 1270/cm can be related to CH₂ bands in non-ionic surfactant-based vesicle and the polylactic acid chain. The particle size and particle size distribution of products were determined by laser light scattering. In fact, due to the high surface to volume ratio of the nanoparticles, they can agglomerate at their surfaces than the bulk ones. The results showed that this reason can be the effect on the size of the product, therefore Di 90% calculated 862.47 nm for NaF nanoparticles loaded on polylactic acid nanoscaffolds. The DLS result was consistent with the corresponding SEM and TEM analysis Fig. 5c showed nanoparticles size with the inhomogeneous particle size of about 100–500 nm.

The Atomic Force Microscope (AFM) as an analysis can be used for studying the topography of materials and biological samples with a scale size ranging from microns to sub nanometers. The AFM images of the final product display which narrow bulge (nanoscaffolds) formed

regularly and along each other, also nanoparticles which have been planted on the surface of the substrate (NaF nanoparticles). The roughness and surface properties of the final products can be calculated with the root mean-squared roughness (RMS) as a factor which obtained from the standard deviation of the AFM image data and determined according to follow equations (Eq. 4):

$$R_{rms} = \sqrt{\frac{\sum_{n=1}^N (z_n - \bar{z})^2}{N - 1}} \quad (4)$$

In this equation z_n represents the height of the n th data, $\bar{z}$ is equal to the mean height of Z_n in AFM topography, and N is the number of data. For an area of 62.12 μm² the roughness average is 23.32 nm. AFM topography images of NaF nanoparticles loaded on polylactic acid nanoscaffolds shown in Fig. 6a. The thermogravimetry analysis (TGA) can be calculated mass change under programmed condition temperatures. Therefore, TGA is mainly used for understanding certain thermal events such as absorption, adsorption, desorption, vaporization, sublimation, decomposition, oxidation and reduction. TGA diagram of the NaF nanoparticles loaded on polylactic acid nanoscaffolds is shown in Fig. 6b. polylactic acid nanoscaffolds as biocompatible and biodegradable polymers can act as a substrate for temperature stabilizer of the NaF nanoparticles. It

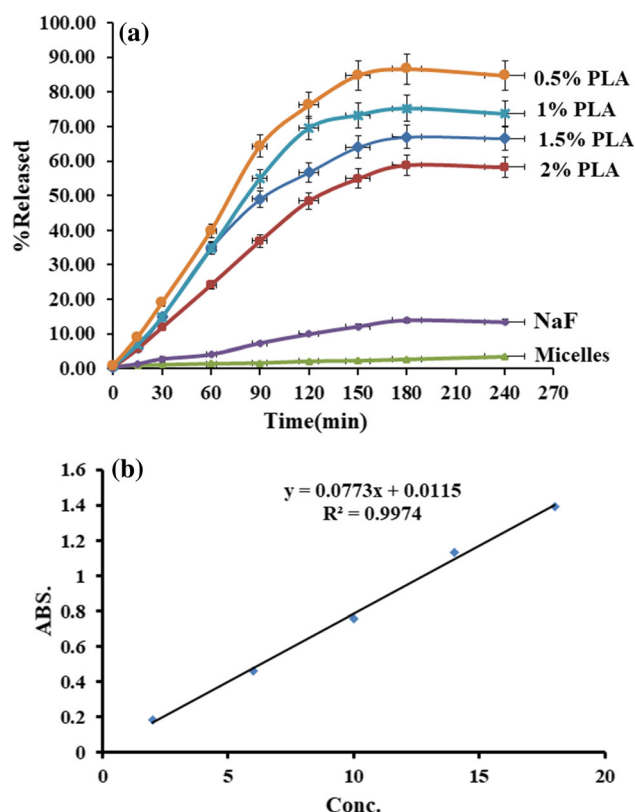


Fig. 7 Cumulative drug release from with different concentrations (0.5%, 1%, 1.5% and 2%), micelles and free drug solutions (pH 7.4), at 37 ± 1 °C (a) and the calibration curve of The NaF encapsulated in PLA nanoscaffolds (b)

can be seen that the thermal behavior of the final product can be divided into two separated regions. The first one in the temperature range 50–250 °C is related to thermal decomposition of the NaF nanocrystals, the weight loss is about 13.48%. The most significant weight loss (mass change: 93.66%) is due to the complete thermal degradation of the nanoscaffold structures which happen in the temperature range 270–340 °C.

In Vitro Drug Release Studies

Vertical all glass Franz-type diffusion cell with an active surface area of 3.46 cm² and a receptor phase volume of 15 ml was used for In vitro release profile study of NaF nanocrystals from polylactic acid nanoscaffolds with different concentrations (0.5%, 1%, 1.5% and 2%), micelles and free drug solutions at 37 ± 1 °C and pH of 7.4 were calculated within 240 min. The membrane (Visking tube, MW cut-off 12,000 D) was used as barrier between donor and receptor compartments of the diffusion cell. The receptor section was filled with normal saline (0.9%) and the donor compartment with 1 ml NaF nanoparticles loaded on polylactic acid nanoscaffolds dispersion in ratio 1:2

deionised water and ethanol. Free drug solutions were used as controls and empty micelles as blanks. One ml samples were withdrawn at fixed time intervals from receptor section and replaced with an equal volume of fresh the receptor medium and the permeated drug concentration was measured. The results in Fig. 7a showed that the polymer concentrations can effect only to release the NaF nanoparticles. Results suggest a sustained release of the NaF nanoparticles from the nanoscaffolds nanomicelles over a period of 240 min. At a time, point of 240 min, the formulation containing 0.5%PLA, 1% PLA, 1.5% PLA and 2% PLA released around 84%, 73%, 68% and 59% NaF nanoparticles from formulations, respectively. This trend shows that the more concentration of the polymer in the formulations, the longer it takes for the nanomicelles to release the drug. This may be attributed to the thick wall which forms with higher polymer concentrations. The calibration curve as a general method calculated for determining the concentration of the substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration. The calibration curve of NaF encapsulated in PLA nanoscaffolds $y = 0.0773x + 0.0115$ with $R^2 = 0.9974$ is described in Fig. 7b.

Conclusion

In this work for the first time, NaF nanoparticles synthesized in micelles and then loaded on polylactic acid nanoscaffolds with coprecipitation assisted microwave method. Final products were characterized by X-ray, SEM, TEM, DLS, AFM and UV–Vis spectrum. Polylactic acid nanoscaffolds act as carriers for NaF delivery. Approximate size of nanostructures was estimated 80–110 nm with Debye–Scherer equation that confirmed by SEM and TEM images. Results show in 0.5% PLA released around 84% NaF nanoparticles from formulations.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and Animal Rights This article does not contain any studies with human or animal subjects.

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