Electrospherization of genistein@DNA core-shell nanospheres as a drug delivery system

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Abstract

The practical application of genistein (GEN) as a drug system is hindered by its low aqueous solubility and poor oral bioavailability. Encapsulation of poor water-soluble drug was considered as one of the widely used approaches to overcome such of these obstacles. The primary goal of this research was to in situ encapsulate the hydrophobic GEN during the electrosynthesis of DNA nanospheres as a delivery system (Es GEN@DNA) with appropriate drug release properties. The prepared Es GEN@DNA nanospheres were characterized using UV-visible spectroscopy, X-ray diffraction analysis (XRD), transmission electron microscope (TEM), zeta potential and stability test. The results revealed that GEN was successfully encapsulate in situ during the DNA electrospherization (Es GEN@DNA) as core shell like structure (Core: GEN and Shell: DNA) with a wonderful stability against time. Furthermore, the drug encapsulation % was studied. In addition, the drug release efficiency of Es GEN@DNA was recorded and theoretically visualized to understand the mechanism and kinetics of GEN drug release. %Encapsulation of GEN within DNA nanospheres was found to be 89.62%. Es GEN@DNA release profile explored that the well entrapped GEN within the DNA nanospheres could be a promising for sustained drug release. Besides, we overcome the dilemma of using a fractal or fractional kinetics model by introducing a general fractional kinetic equation that involves a time-dependent rate coefficient, which introduced that the solution of the fractional kinetic model is capable of fitting the release data profiles of free GEN and Es GEN@DNA.

Introduction

Genistein (GEN) is one of the main aglycone forms of isoflavones found in soybean and soy products [1], received growing interest around the world in the last decade. It is commonly used as a drug in pharmaceutical industry or as a nutraceutical ingredient in the food industry [2]. Many studies have been proven that GEN has beneficial health effects, such as lowered rate human cancers [3], reduced the incidence rate of cardiovascular diseases [4], obesity and diabetes and bone health improvement [5]. Also, it would be a great advantage to add genistein to food products [2].

The introduction of GEN as one of a class II agents in the biopharmaceutical classification system into drug in pharmaceutical industry is hindered by a number of technological challenges due to their sensitivity to light, heat, and oxidation [6]. In addition, GEN shows extremely low water solubility and bitterish taste, which limits its pharmaceutical applicability [7]. Furthermore, genistein's poor absorption and rapidly metabolized after oral administration strongly limit its bioavailability [8]. Due to all of the above mentioned inherent drawback of GEN, there is an urgent need for an economical and feasible delivery system to enhance the solubility and dissolution rates, and bioavailability of genistein while maintaining its chemical stability, and controlling its release[9].

Different methods have been introduced to promote the bioavailability of GEN. These include approaches such as micelles formation[10], sonochemical [11], nanosuspension formulation [12] and emulsifying [13]. Such methods have some limitations such as imperfect solubility and release of drugs in carriers,
relatively higher surface area, and in some cases, carrier matrix incorporates the drug in the amorphous form at elevated temperatures, which make the overall system unstable [13].

Recently, the growing development of the nanomaterials technology has revolutionized the map of the drug delivery systems [14]. Among the various nanomaterials technology, an emerging platform called structural DNA nanotechnology, which considered as a subfield of spherical nucleic acid (SNA) [15]. DNA nanotechnology has attracted the attention of the scientists as a valuable bioengineering material which opened new horizons in many application fields. This exceptional promise of DNA technology was due to availability, biocompatibility, biodegradability, sustainable, adaptable material, no toxicity, transparent and inexpensive material [16]. In addition to its self-assembling and engineering capabilities with high accuracy and precision [17].

Several studies have introduced DNA molecule as a potential nano- and micro- building structures which utilizes for the overcoming most of the current limitation of the delivery systems such as a delivery of the poorly soluble drugs[18]. Chemically, the double helical strand DNA (dsDNA) considered as thread-like polymer. Deoxyribonucleotides were the building monomers of the DNA chains, which basically consist of a nucleobase-pentose sugar-phosphodiester group[19]. DNA backbone have the interaction ability with the positively charged species because of the negatively charged hydrophilic phosphate groups and sugar molecules [20]. In addition, the aromatic bases have the binding affinity with the negatively charged species. This many-sided nature promotes the participating of DNA in the strong competition for molecular self-assembly [21, 22].

Moreover, DNA counted as a vital biological polyelectrolyte. In aqueous solution, DNA backbone becomes negatively charged due to the presence of phosphate groups. So, it characterized as a suitable matrix for the solution-based nanomaterials formation [23].

As a part of our on-going research, the aim of the present study was to create a cost-effective delivery system for poorly soluble genistein that keeps its chemical stability and controls its release to be a promising drug delivery system. To the best of our knowledge, no information is available on the electrospherization of DNA molecules as a GEN drug nanocarrier.

UV-visible analysis, X-ray diffraction (XRD), transmission electron microscope (TEM), size distribution, Zeta potential analysis and stability test were conducted to explore the characteristics of the prepared materials. Additionally, % drug entrapment efficiency (%EE) was examined for the optimized formulation. The formulation was studied for in vitro drug release, and theoretically the drug release data was examined using different theoretical models to explore the kinetics and the behavior of the GEN release. We hope this study will be beneficial to further manipulate for utilization of DNA nanospheres to enhance solubility and bioavailability of poorly water-soluble drugs.

**Materials And Methods**

**Materials**
Deoxyribonucleic acid; sodium salt, type III: from salmon testes (dsDNA), two platinum rectangular sheets (Pt), dimethyl sulfoxide (Cas No. 67-68-5) and dialysis tubing (molecular weight cut off 12–14 kDa) were purchased from Sigma Aldrich, St. Louis, MO, USA. Sodium hydroxide (NaOH) was purchased from PanReac AppliChem, Darmstadt, Germany and deionized water (resistivity > 2×10^8 Ω cm). Genistein was procured from DSM nutritional products Ltd., Switzerland, Germany. All other reagents were of analytical grade and used as received.

**Electrospherization Of Dna Nanospheres (Es Dna)**

The electrospherization of DNA was performed using electrochemical method. The electrolyte solution was prepared by following the procedure described by us earlier for pure DNA nanospheres \[24\]. Briefly, a 0.2 wt. % of pristine DNA aqueous solution was prepared by dissolving DNA in deionized bi-distilled water under ambient condition. Next, a fresh prepared solution of NaOH was added to a previously prepared DNA aqueous solution (10 ml) to adjust the pH at 12. The resultant solution was stirred for 1h to allow complete reaction. After this time, the electrochemical cell which consists of two platinum sheets 5 cm apart was dipped vertically in the DNA electrolytic aqueous solution. Immediately afterwards, a constant potential of 2.5 V (using potential power supply; ECOS) was applied. The reaction procedure was carried out under magnetic stirring (200 rpm.) for 6 h at the ambient condition. The final electrospherically treated solution named Es DNA. Besides, to further investigate the role of the electrospherization time on the reaction solution, the procedure was performed for different time intervals 1, 2, 3, 4, 5 and 6 h.

**Electrospherization Of Genistein Loaded Dna Nanospheres (Es Gen@dna)**

GEN loaded DNA nanospheres were prepared using our previous method \[24\], a fresh solution of the alkaline treated DNA aqueous solution was prepared followed by adding 0.1 wt.% of GEN. (it was selected as a medium in view of the poor solubility of the drug in water). Next, the electrochemical preparation at constant potential 2.5 v for 6h was performed. The resultant solution (labeled Es GEN@DNA) was centrifuged at 21,000 rpm; 1 h at 4ºC using the cooling centrifuge (Beckman Model J2-21 centrifuge, California, USA) to separate the prepared DNA nanospheres from the free drug and excess surfactant. The separated DNA nanospheres were washed using purified water of Milli-Q quality three times. Finally, the resultant nanospheres were used immediately for analysis or lyophilized (Freeze dryer Gold-SIM, FD8-8T, USA).

**Characterizations**

**UV-visible spectra measurement**
The characteristic behaviour of pristine DNA aqueous solution, the influence of the electrospheriezation time on the reaction solutions (Es DNA and Es GEN@DNA), and the effect of the storage time on the prepared nanospheres stability were examined using the UV-vis spectrophotometer (T80 double beam model, pg 18 instruments-UK). The recorded patterns covered the wavelength range of 190–1100 nm with ± 0.3 nm accuracy.

**Transmission Electron Microscopy**

To study the morphology and distribution of the prepared solutions, TEM images of the prepared DNA nanospheres with and without the presence of the genistein were collected using TEM (JEOL TEM-2100) connected to a CCD camera. Firstly, the solutions were sonicated using ultrasonic cleaner; model XH-E412, then a drop of the prepared solution was placed onto a carbon supported Cu grid followed by the solvent evaporation before capturing the TEM images.

**Zeta Potential Experiments**

DNA surface charge was due to the existence of the phosphate groups on the backbone of its structure, so measuring the zeta potential will be helpful in the imagination of nanospheres formation mechanism. Zeta potential analysis was performed using Zetasize Nano-zs90 (Malvern instruments, Malvern, UK) on a freshly as prepared DNA solution. Surface charge distribution was recorded three times at room temperature.

**X-ray Diffraction Analysis (Xrd)**

XRD patterns of Es DNA, GEN, and Es GEN@DNA were collected using an D8 advance X-ray diffractometer with Cu Kα (1.5406) as a radiation source (Bruker, Germany) to investigate the structural nature of the GEN, lyophilized EsDNA, and Es GEN@DNA. The anode X-ray tube were set to at applied voltage 40 kV and current 40 mA. The samples patterns were recorded over 2θ range from 30-50° at a 0.03/sec step.

**Stability Test**

The stability of the prepared samples against aging and aggregation was examined by measuring the UV-vis. Patterns using UV-vis. spectroscopy for different storage time extended to at least 2 months.

**Characterization Of The Es Gen@dna**

**Determination of encapsulation efficiency and release kinetics**
The drug content within nanospheres was quantified by an UV–vis spectrophotometer. The un-encapsulated drug was separated from the prepared nanospheres by centrifugation at condition 13000 rpm for 60 min. and filtrated using 15 kDa Amicon Millipore filter tubes. Afterward, 2 mL of the nanosphere suspensions were digested with equal volume of DMSO followed by the filtration through 15 kDa Amicon filter tubes. The drug encapsulation efficiency (%EE) in nanospheres was calculated from a calibration curve made for the respective drug in the range of 1–9 µg/ mL. The % EE was calculated using the following equation:

\[
\%\text{EE} = \frac{\text{Drug in nanospheres}}{\text{Total drug added}} \times 100 \quad (\text{Eq. 1})
\]

Furthermore, the cumulative drug release pattern from the prepared nanospheres was conducted by dialysis method. Here, 3 mL of nanosphere solution was enclosed in the dialysis tube of 12,000–14,000 Da and placed into phosphate buffer saline pH 7.4 (50 mL) medium under 100 rpm stirring using a shaking incubator (GFL Gesellschaft für Labortechnik, Burgwedel, Germany) at 37 ± 0.5 °C. The process was conducted up to 48 hrs, 50 µL volume of sample was drawn from the dialysis bag using a 0.45m pore size syringe filter. Immediately afterward, replaced with fresh dissolution medium to keep sink state. The drawn amount of respective drug was assessed by comparing the readings from UV-vis. Pattern with the calibration curve at 265 nm. Under the same conditions, Free GEN release was considered as a control.

**Theoretical modeling of the release profiles of free GEN and Es GEN@DNA**

Different theoretical models are suggested for evaluating the mechanism and kinetics behavior of GEN drug release.

**Results And Discussion**

**UV–visible spectra analysis**

The UV-vis pattern of the aqueous solution (pH 7) of DNA was recorded as shown in figure 1, which shows the absorption peak maxima of DNA at 240 nm due to the π-π* absorption transition of aromatic base molecules[25].

**Effect of electrospherosis reaction time**

It has been widely accepted that electrosynthesis time has a crucial role on the preparation of the nanomaterials[24]. In order to confirm the formation of the DNA nanospheres during electrospherosis process in the prepared aqueous solution, the characteristic absorption analysis was conducted for the as-synthesized DNA nanospheres Fig. 2. The course of synthesis was followed by acquiring the respective absorption spectra at various stages, as depicted in Fig. 2a. the UV-vis patterns explored a
progressive increase in the absorption intensity around 299–315 nm as a function of the electrospherization time. This was accompanied by a new growing peak emerged around 372–500 nm but takes long time to emerge[24].

The UV-vis absorption pattern of Es GEN@DNA solution was collected and compared with that of Es DNA for the prepared as depicted in Fig. 2b., we can recognize the formation of DNA nanospheres in the presence of genistein.

In depth, fine details were clarified using the curve deconvolution as explored in Fig. 3. we noticed that the area under the curve at wavelength 400 nm of the GEN incorporated DNA NPs sample was higher than that of native DNA nanospheres, this may be assigned to that GEN promotes the ability of DNA nanospheres formation in compared with Es DNA. On the other side, these results suggest that the drug may be incorporated within the formed spheres[24].

Next, conducting the zeta potential in order to gain new evidence in support of this assumption was an urgent need.

**Zeta potential study**

<table>
<thead>
<tr>
<th>Sample name</th>
<th>DNA</th>
<th>Es DNA</th>
<th>Es GEN@DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\zeta$-potential (mV)</td>
<td>-31.4</td>
<td>-17.4</td>
<td>-14.3</td>
</tr>
</tbody>
</table>

It is also remarkable that the zeta potential measurements introduced support to the thought of the spherization of DNA molecules[26]. Table 1 represents the value of $\zeta$-potential in mV of the native DNA molecules, electrospherized DNA with (Es GEN@DNA) and without (Es DNA) GEN drug. -31.4 mV was the measured zeta potential value of DNA solution [19, 27]. After the electrical perturbation applied to the DNA solution, the negative value of the zeta potential was considerably reduced to value of -17.4 mV (Table 1). Accordingly, the number of the phosphate groups directed towards the solvent decrease, which in turn reducing the ability of the DNA chains to uptake water molecules [28].

This behavior supports that phosphate groups may be participate in the development of the DNA nanospheres, where the polymer chain has the ability to turn its conformation by means of intersegment interactions and/or its tendency to the adjacent solvent molecules.

In depth, there are an opportunity for the DNA chains to collapse into spheres because of the attractive and hydrophobic interactions [28]. On the other hand, the zeta potential value of the Es GEN@DNA solution was −14.3 mV (Table 1), giving an indication of good electric repulsion and stability. This remarkably in coherence with the above results and encourages to predict that the negative charged phosphate groups may be directed inwards the DNA nanospheres[29]. Consequently, this could be
considered as good evidence that there are an electrostatic and hydrophobic interactions at the segment scale of DNA chains includes the phosphate groups [30–32].

**Morphology and size distribution study using TEM**

The shape and particles size of the DNA nanospheres was further confirmed using TEM measurements.

Figure 4 represents the captured TEM images and the drawn size distribution of the electro-prepared nanospheres Es DNA, which explored the formation of approximately nano spherical structures Fig. 4(a1) with wide size distribution as demonstrated by UV-vis patterns. the average size $D_{av}$ of the Es DNA nanospheres was 76.5 nm as depicted by the size distribution curve Fig. 4(a2).

Figure 4 (b1) and 5 introduce the TEM images of Es GEN@DNA sample, under the perturbation of the applied voltage of DNA/GEN solution, TEM images clearly explored the formation of roughly spherical structure, which have an apparent core-shell like structure. In depth, GEN drug uniformly distributed as a core with good, homogeneous incubation within the formed DNA shell [84]. The size distribution curve of Es GEN@DNA introduced that the uniform and compact core-shell like nanostructure have an average size of 109.2 nm as depicted in figure 4 (b2).

**XRD analysis**

Generally, X-ray diffraction (XRD) analysis is a non-destructive technique that used to examine the crystallinity and physical nature of the nanospheres [33]. XRD patterns of Es DNA, Pure GEN and Es GEN@DNA were acquired and compared the significant differences in the molecular state of the nanoformulation. The XRD pattern of pure GEN showed some intense peaks corresponding to Bragg angles ($2\theta$) at 7.5°, 12.1°, 12.8°, 14.2°, 14.8°, 16°, 16.5°, 18°, 19.2°, 20.9°, 22.4°, 23.2°, 26.3°, 27.3°, 28.6°, 29.4°, 33.5°, 36.1°, and 40°, representing crystalline nature of GEN[6]. Interestingly, GEN lost its crystalline phase in the Es GEN@DNA sample. These changes in the property indicates GEN was successfully assimilated into DNA in an amorphous nature. It can be concluded that during the electrosynthesis of the DNA nanospheres in the presence of the GEN drug, there was no sufficient space for GEN molecules to develop a crystalline structure.

**Entrapment efficiency %EE of GEN**

Besides, %EE of GEN in DNA nanospheres was found to be 89.62%. This high %EE suggested that the formation of core/shell structure which could be due to high hydrophobic interaction between GEN core and DNA shell. The stable nanosized compact core/shell like structure particles and high %EE value could be helpful in sustain GEN release profile [34].

**Drug Release profiles**
Subsequently, the releasing profile of free GEN and Es GEN@DNA at pH 7.4 for 48 h were investigated to evaluate the release profile of genistein. As depicted in Fig. 7, the release of drug alone was relatively rapid (~ 70% release) for up to 2 h, and later more release (about 100%) in the next 24 h. On the contrary, the release of GEN@DNA was found to be slow (~ 30% a burst release during initial 2h) followed by constant release during the last 48 h. In details, in vitro release profile of Es GEN@DNA presented a typically biphasic release pattern. An early burst release occurred due to the poorly entrapped GEN within DNA nanospheres and just situated underneath the outer edge of the nanospheres, which was definitely diffused through the dialysis membrane to the release medium. Then, a continuous sustained release of GEN was denoted for more than 48h, which assigned to the well entrapment of the drug within the DNA nanospheres. The subsequent release of the drug from the nanospheres could be theorized as a diffusion of the drug from the delivery system to the release medium, or by the erosion of the delivery vehicle. It actually depends on the morphology, constituents, surface properties of the nanospheres and the physicochemical features of the molecules. Finally, the obtained drug release profile confirmed valuable in slow and sustained release to realize better therapeutic efficacy in disease treatment.

**Time Stability**

Stability was considered one of the valuable factors which should be investigated to examine the possible application of the prepared nanospheres as a drug carrier. The time stability of the prepared Es GEN@DNA nanospheres was studied using UV-vis to examine their preservation in aqueous environment against aging and aggregation. As shown in Fig. 8, UV-vis patterns revealed that Es GEN@DNA nanospheres could last at 4°C for over approximately two months [19]. This result was in consistent with the zeta potential analysis.

**Mechanism And Kinetics Of Gen Drug Release**

Theoretically, various models are used for evaluating the GEN drug release data to realize the behavior and kinetics of GEN drug release. For instance, the Peppas equation (power-law model) [35], the Weibull model (stretched exponential) [36], zero-order kinetic model[37] and the first-order kinetic model [37]. In various situations, such as in complex media or heterogeneous medium, the previous kinetic models are not appropriate to describe the diffusion of drugs corresponding to experimental data of tracking particles. Therefore, alternative models have been developed via fractional differential equations; for more details, see Ref. [36]. The fractional differential equation with fractional derivatives can be used instead of considering a time-dependent rate as in fractal kinetics. Here we attempt to generalize the classical and fractional models studied in pharmaceutical literature through a fractional kinetic model that includes a general form of time-dependent rate. Such fractional equation and its approximation have been shown to describe the experimental data of the slow diffusion process [38]. Moreover, we hope this study will be more beneficial to further manipulating for utilization of DNA nanospheres to enhance the solubility and bioavailability of poorly water-soluble drugs.
General fractional kinetic model

Different kinetics models have been presented to study the analysis of data sets of various drug processes. One of the most common approaches is the so-called first-order process, which is widely used to study a non-exponential formalism described by a power-laws and is given by [35]

\[
\frac{\partial (R_0 - R(t))}{\partial t} = -k(t)(R_0 - R(t)).
\]

Here \(R\) is the drug concentration, \(R_0\) is the saturation solubility and \(k(t)\) is the time-dependent rate coefficient. In the view of anomalous kinetic description, we generalized the first-order process into the fractional form with keeping a general form of time-dependent rate instead of a power-law rate coefficient suggested in Ref. [39]. Therefore, Eq. (1) can be considered as

\[
\left( \frac{1}{\varphi(t)} \frac{\partial}{\partial t} \right)^\alpha (R_0 - R(t)) = -k_\alpha (R_0 - R(t)),
\]

where \(k(t) = k_\alpha \varphi(t)\), \(k_\alpha\) is the rate coefficient in respect to fractal dimension and \(0 < \alpha < 1\). The time-fractional derivative above is the so-called regularized Caputo fractional derivative, which is defined as [40]:

\[
\left( \frac{1}{\psi'(x)} \frac{\partial}{\partial t} \right)^\gamma f(x) = \frac{1}{\Gamma(1 - \gamma)} \int_0^x d\xi (\psi(\xi) - \psi(x))^{-\gamma} \frac{\partial f}{\partial \xi}.
\]

From Eq. (2) with follow the procedure used in [41, 42], we obtain

\[
R(t) = R_0 \left( 1 - E_\alpha \left[ -k_\alpha (D(t))^\alpha \right] \right),
\]

where,

\[
D(t) = \int_0^t d\tau \varphi(\tau)
\]

and \(E_\alpha\) is the Mittag-Leffler function defined as [42],

\[
E_\beta(x) = \sum_{n=0}^{\infty} \frac{x^n}{\Gamma(1 + \beta n)}.
\]
It is worth noting that the chosen values of the fractional order $\alpha$ with a specific form of the rate coefficient led to the familiar kinetics models (classical and fractional models) given in the literature. And we pursue that topic more clearly in the following cases.

**Case I: Constant rate**

In this case we consider a constant rate, i.e., $k(t) = k_\alpha$, then Eq. (3) is reduced to

$$R(t) = R_0 \left(1 - E_\alpha [-k_\alpha t^\alpha]\right).$$

Also, the zero-order kinetic model can be considered as a particular case of Eq. (4) at the small argument in the case of $\alpha = 1$, which leads

$$R(t) / R_0 = k_0 t.$$

**Case II: Power-law rate**

In this case, we consider the rate coefficient is given by a power-law form, i.e., $k(t) = k_\alpha t^{-\sigma}$. Therefore, Eq. (3) can be written as

$$R(t) = R_0 \left(1 - E_\alpha \left[-\frac{k_\alpha t^{\alpha(1 - \sigma)}}{(1 - \sigma)^\alpha}\right]\right).$$

Moreover, in case of $\alpha = 1$, Eq. (5) is reduced to the familiar form of the so-called Weibull model (stretched exponential model) [36].

$$R(t) = R_0 \left[1 - \exp \left(-\frac{k_0 t^{1 - \sigma}}{(1 - \sigma)}\right)\right].$$

which is usually used in drug release studies despite its extensive empirical use in dissolution studies. In addition to that, a classical power-law kinetic model can be observed from Eq. (6) at short times, which leads to

$$R(t) / R_0 = k t^{1 - \sigma}, \quad k = k_0 / (1 - \sigma).$$

**Case III: Exponential rate**

In this case, we choose the rate coefficient in exponential form, i.e., $k(t) = k_\alpha e^{-\gamma t}$, therefore one can obtain the drug release through Eq. (3), which is given by
This model represents a new hypothesis that will be discussed in the next section.

Figure 9 and 10 showed the fitting curves for release profiles of GEN and GEN@DNA in phosphate buffer saline, pH 7.4, via the different kinetics models i.e., fractional kinetics models with exponential, power-law, and constant rate. Our illustration is based on choosing an arbitrary value of the time-fractional order “α”, coefficient rate “κ_α” and σ, γ exponents. Moreover, from Figs. 9 and 10, one can find that the new fractional kinetic consideration Eq. (6) (fractional kinetic equation with the exponential time-dependent rate) is the most capable in describing the release profile of free GEN and Es GEN@DNA in phosphate buffer saline.

In Fig. 11, we compared the three kinetics models (models with exponential, power-law, and constant rates) represented by equations (4), (5) and (6) respectively for two different values of the time-fractional order “α” at κ_α = 1 and σ = γ = 0.5, which showed that the superior of the fractional exponential rate model due to the long relaxation release especially with decreasing the value of the time-fractional order. In addition to that we illustrated the effect of the exponents σ and γ on the release behavior for the exponential and power-law models given by equations (5) and (6). And Fig. 12 showed that increasing the value of γ in the exponential rate model led to fast saturation point instead of that described with the power-law or constant rate models. Therefore, from our theoretical study with the fitting of datasets, we conclude that, the time-fractional order with the so-called inverse relaxation exponent γ can controls the release saturation corresponding to the heterogeneity of the medium with a memory effect (slow diffusion of the drug in a medium).

**Conclusion**

This study introduced a facile one-step and cost-effective process to in situ entrapment of GEN within DNA nanospheres as core shell like structure using electrosynthesis approach. The results of UV-vis, XRD, TEM, zeta potential and stability test approved that in the presence of GEN, DNA nanospheres can be formed easily and promoted the concentration of the formed nanospheres, which have well defined core/shell like structure with broad size distribution explored that the average size D_{av} of the prepared DNA nanospheres was 109.2 nm. The lessening in the surface charge of DNA after electrospherization supported the idea of the tendency of the DNA chain to compact into sphere structure. On the other hand, XRD introduced that GEN entrapped within DNA nanospheres matrix lost its crystalline nature. Shortly, a well-defined uniformly distributed Es GEN@DNA nanospheres with a stability for at least 2 months at 4°C have a good, homogeneous incubation of GEN within the formed DNA nanospheres. Besides, % encapsulation and drug release profile explored that GEN was highly entrapped with 89.62% in addition to a promising sustained release profile. Finally, the theoretical studies based on the comparison between the fractional analysis with the experimental release data, we found that the fractional model with the
exponential coefficient rate is the most capable of describing the release profile of free GEN and Es GEN@DNA.

**Abbreviations**

genistein: GEN; Deoxyribonucleic acid: DNA; spherical nucleic acid: SNA; double helical strand DNA: dsDNA; % Drug entrapment efficiency: %EE.

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and material**

During this study, all data produced or evaluated are involved in this published article.

**Competing interests**

The authors declare no competing interests.

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**Authors' contributions**

**Conceptualization:** Ayman and Fikry, **Methodology:** Aya and Ayman and Fikry, **investigation:** Aya and Ayman, **Analysis:** Aya, Amira and Ayman, **theoretical analysis:** Ashraf, **writing original draft:** Aya, Amira, Ashraf, Ayman and Mohamed. **Writing review and editing:** Aya, Amira, Ashraf, Ayman and Mohamed. All authors have read and agreed to the published version of the manuscript.

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Last, we would like to introduce our respects to our co-author Prof. Mohamed M. Gabr, he is taking the responsibility of our laboratory (Biological Advanced Materials laboratory) after prof. Fikry passed away.
References


Figures

Figure 1

Uv-visible spectra of dsDNA
Figure 2

(a) Uv-visible spectra of Es DNA as a function of time, (b): Uv-visible spectra of Es DNA and Es GEN@DNA after electrospherization for 6 h.
Figure 3

Curve deconvolution of Uv-visible spectra of Es DNA and Es GEN@DNA spectra after electrospherization for 6 h.
Figure 4

TEM images of (a1) Es DNA, (b1) Es GEN@DNA and the corresponding size distribution curve of (a2) Es DNA, (b2) Es GEN@DNA
Figure 5

TEM image represents the incorporation of the drug within the formed nanospheres
Figure 6

XRD patterns of GEN, Es DNA and Es GEN@DNA.
Figure 7

In vitro cumulative release profile of GEN and Es GEN@DNA in phosphate buffer saline, pH 7.4.
Figure 8

UV–vis pattern of Es GEN@DNA against storage time at 4°C.

Figure 9

Three plots corresponding to the datasets of In vitro cumulative release profile of GEN in phosphate buffer saline, pH 7.4. together with the best fitted curves (Exponential model (a), Power-law model (b), constant model (c)).
Figure 10

Three plots corresponding to the datasets of In vitro cumulative release profile of Es GEN@DNA in phosphate buffer saline, pH 7.4. together with the best fitted curves (Exponential model (a), Power-law model (b), constant model (c)).

Figure 11

Comparison of the three different kinetic release model for two different values ((a) \(\alpha=1\) and (b) \(\alpha=0.5\)) of fractional order.
Figure 12

Comparison of the exponential model for different values of \( \gamma \) and power-law model for different values of \( \sigma \).