



Development and *in vitro* assessment of Norfloxacin nanoparticles

Bandar E Al-Dhubiab*

Department of Pharmaceutical Sciences,
College of Clinical Pharmacy, King Faisal University, Al-Ahsa, Saudi Arabia.

Abstract

Norfloxacin, a fluoroquinolone that directly inhibits the bacterial DNA synthesis, is frequently used in the treatment of urinary infections caused by gram-negative bacteria. Nanoparticles present a viable alternative for effective bioavailability for both prolonged durations and systemic effect, thereby increasing medication compliance and efficiency. The objective of this study was to formulate gelatin nanoparticles of norfloxacin using the Buchi nano-drying method and evaluate *in vitro*. Norfloxacin nanoparticles were analyzed for size and scanned for surface morphology. Zeta potential, product yield and *in vitro* release pattern were also assessed. Formulation yield was at 79.5%, while product recovery was 65.6%. Surface morphology indicated the particle surface was smooth whereas size distribution was bimodal. Results from *in vitro* release pattern demonstrate a biphasic release pattern that was 35% at 30 min, with a prolonged release of 97.6% that went up to 12 h. Differential scanning calorimetry studies signifies the change in drug state from crystalline to amorphous. In conclusion, the data observed here indicates the potential of developed nanoparticles to provide controlled release and could be a feasible alternative for antibiotic delivery systems.

Keywords: Gelatin, morphology, nano-drying, particle size, controlled release

INTRODUCTION

Norfloxacin is a fluoroquinolone with broad range of activity against both gram-positive and gram-negative aerobic bacteria, with limited activity against anaerobes [1]. It is a second-generation antibiotic derived from nalidixic acid, used as a first-line drug for the treatment of diarrhea, neutropenia, bacteria induced gastroenteritis, gonococcal and urinary tract infections [1, 2]. Pharmacologically, norfloxacin is often administered for long-term purposes. However, the low bioavailability of this drug makes administration challenging [3]. These two factors, in addition to the drug concentration at the site of action are key factors in the determination of drug dosage. As such, norfloxacin is often administered via a frequency-dependent dosing regimen. The wide range of indications for norfloxacin and its effectiveness is reflected in its frequent administration for common illnesses like urinary tract infections [4]. However, the frequency dependent dosing regimen needed to reach steady state drug levels lend itself to issues of drug compliance among patient populations. As such, there is a demonstrated need for a targeted drug delivery system for a widely-used antibiotic like norfloxacin. Oral delivery of drugs present the most frequent method of drug administration, but has several challenges. One of the main challenges with the administration of norfloxacin is the frequency dependent dosing that requires continued oral delivery to maintain therapeutically useful levels for metabolism in the body. A solution to this problem would be a targeted drug delivery system that can prolong drug delivery either via extended release or one that reaches high therapeutic steady states intrinsically. Both methods would lead to a reduction in the dosage frequency administered to patients. Nanoparticle research is currently the mainstay in the pharmaceutical industry, with the goal of creating novel drug delivery systems [5]. Norfloxacin loaded gelatin nanospheres were prepared using the fourth-generation lab Nano spray dryer by Buchi [6]. The pilot study in which norfloxacin loaded biodegradable nanoparticles were created, is an attempt at creating an alternative drug delivery system that is targeted and site specific, thereby eradicating unwanted side-effects in the process.

There are several techniques employed in the field for the formulation of nanoparticles, though this study employs the spray-drying method. The spray-drying method is a single-step, rapid and easily scalable method used in the conversion of liquid into solid in the form of dry powder [7]. As such, this method serves a

more cost and time-efficient alternative to the multi-step and time consuming aqueous dispersion methods [8]. Solid-liquid nanoparticles formulated using the conventional method are typically not stable, use toxic solvents in the process and are therefore not safe for human consumption [9]. Toxicity is the same caveat that applies to the solvent diffusion and water-in-oil methods for nanoparticle formulation. The objective of this study was to prepare, characterize and evaluate the potential of norfloxacin loaded gelatin nanospheres to target the gastrointestinal system.

MATERIALS AND METHODS

All chemical and reagents used in experiments were of analytical grade except for norfloxacin hydrochloride (Sigma, St. Louis, Mo), gelatin bloom 300 (Fisher Scientific (Loughborough, UK).

Formulation of nanoparticles

Nano-drying method (BUCHI Labortechnik AG, Switzerland) was employed to prepare norfloxacin polymer-loaded nanoparticles as previously described [10]. However, in this paper, some modifications were made to the methodology. Briefly, a spray nozzle of 4 μm was used with a relative fixed spray rate of 100% and a flow rate of 100 L/min. Temperature of both inlet and outlet was set at 120 °C. Polymer (gelatin 500 mg) was soaked in water for 30 min and pre-warmed. Equal amounts of gelatin and norfloxacin were dissolved and then filtered to avoid the blockage of the nozzle and then spray dried. Afterwards, dried particles were scraped from the chamber, collected and stored in a desiccator at 25 °C [11].

Drug content

The percentage of norfloxacin in prepared nanoparticles was determined after dissolving. Briefly, the nanoparticles was mixed with water by continuous shaking on a water bath at room temperature for 12 h. Then heated for 10 min followed by cooling, and diluted to 25 mL with distilled water [12]. The solution was analyzed spectrometrically at 273 nm (UV-1601 Spectrophotometer, Shimadzu).

Particle size analysis

Nano-ZS Malvern Nano Series, (Malvern Instruments Private Ltd, MA, US) was used to determine the particle size. A small volume of nanoparticles suspended in phosphate-buffered saline was transferred into the glass cuvette of the instrument (2 mL). From this, the mean size diameter and distribution of nanoparticles were determined [13].

Zeta potential

The zeta potential or effective electric charge of the nanoparticles was calculated. This value was calculated using the Nano-ZS Malvern Nano Series (Malvern Instruments Private Ltd, MA, US) [14].

Scanning electron microscopy

The external surface of the nanoparticles was examined for any changes in structure such as cracks, hollows or change in the shape. Spray-dried nanoparticles were coated with a thin layer of platinum sputtering. Afterwards, surface morphology was determined using the Quanta 200 scanning electron microscopy (FEI/Philips, Eindhoven, Netherlands). Technical triplicates were carried out to determine average values [15].

Product yield

Product yields were calculated from spray-dried powder that was collected via scraping from a collecting vessel. Powder was weighed and the product yields were calculated as the difference between the total amount of solid sprayed, S1 and the weight of the recovered nanoparticles, S2 using below equation [16].

$$\text{Product Yield} = \left(\frac{S2}{S1} \right) \times 100$$

Drug release pattern

In-vitro drug release patterns were performed by using a water-bath shaker. About 100 mg of the nanoparticles were retained in the dialysis bag (MW cut off: 12-14 kDa, Thermo Fisher Scientific, Inc) and dipped into 250 ml of 0.1 M phosphate saline buffer (pH 7.4). At different time intervals (0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h), a 10 ml sample was removed from the beaker and replaced with 10 ml of saline buffer to maintain the sink condition. The solution was filtered through filter membrane (0.2 μm ; Millipore Corporation, Bedford, MA) and norfloxacin content was analyzed spectrophotometrically at 273 nm using the Shimadzu UV-1601 Spectrophotometer. The amount of norfloxacin released from nanoparticles was determined kinetically by various mathematical models [17].

Stability studies

The nanoparticles were placed in a bottle and stored for 12 months at different temperatures (3 $^{\circ}\text{C}$ –5 $^{\circ}\text{C}$, 15 $^{\circ}\text{C}$ –25 $^{\circ}\text{C}$, and 37 $^{\circ}\text{C}$) and relative humidity (75 \pm 5%), according to ICH guidelines for long-term stability studies [18]. The morphological examination and drug content of the nanoparticles were examined every month.

Data analysis

Statistical analysis was done by t-test using Graphpad prism 5 (graphpad software, Inc., CA). A significance level of $p < 0.05$ denoted significance in all cases [19].

RESULTS AND DISCUSSION

The nanoparticles were successfully prepared by nano-drying method. The norfloxacin content in prepared nanoparticles were 88.52% \pm 5.26%. It is essential to measure the distribution size of formulated particles to optimize the drug delivery system. As such, the determination of the particle size will ensure precise targeting and limit toxicity. To determine the size of the nanoparticles, dynamic light scattering method was used. Unexpectedly, it was found that a bimodal distribution in the particle size curve (Figure 1). The larger portion of the formulation comprised of 79.8% particles which had a diameter average of 502 nm. The second portion, which was smaller, comprised of 8.8% particles, with an average diameter of 5.1 μm . This smaller portion is deal for an initial bolus of loading dose to be followed by a period of longer and sustained release by the larger portion [10, 20].

The surface structure of the norfloxacin gelatin nanoparticles was also examined using imaging technology. It was found that the spray drying method elicited smooth and shrived surface folding (Figure 2). It can be seen from the Figure 2 that no holes or cracks on the particle surface. These attributes ensure efficient long-time and non-fluctuating drug release. The spray droplet was exposed to a higher temperature in the drying chamber which may have influenced surface folding, and resulted in shrinkage, which may be overcome by having the concentration of the polymer increased as described in literature [21].

This study employed the Buchi Nano B-90 spraying method to increase the possibility of producing uniform particles within a small volume of sample. The encapsulation efficiency of obtained particles were found to be at 79.5%, which was superior as compared to conventional methods [22, 23]. However, the percentage of product recovery was calculated to be 65.6%, most likely due to some product loss from sticking to the wall of the drying chamber (Figure 3). It is likely that such loss can be avoided in the future with an increase of product volume up to 4 times than used in the current study, as observed in earlier studies [11, 21]. Further, the zeta potential of prepared nanoparticles were found to be -18 mV.

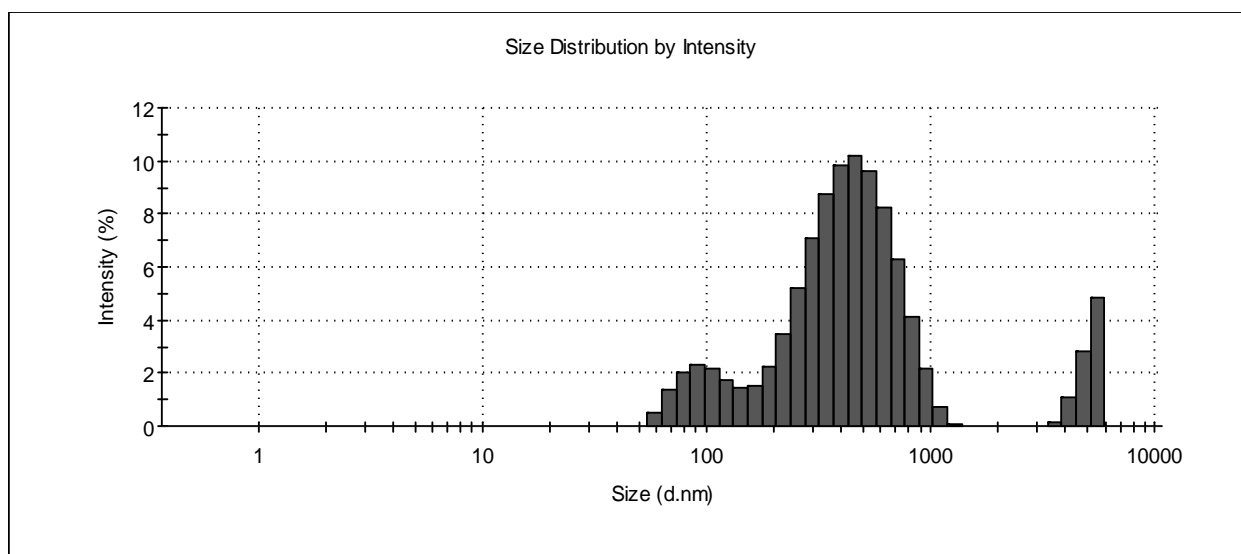


Figure 1: Biphasic particle size distribution of norfloxacin gelatin nanoparticles prepared Buchi Nano Spray-dryer B-90.

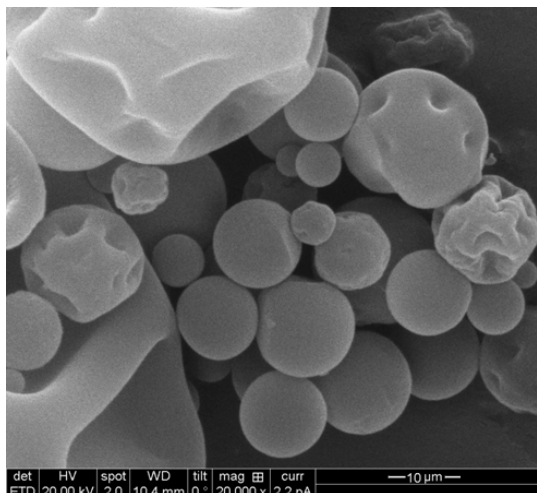


Figure 2: External surface morphology showing shriveled shape prepared by spray-drying method.

The release profile of norfloxacin from the formulation demonstrated a sustained release of up to 12 h, with 97.6 % release. An initial bolus was released within the first 30 min, with a release of 35%. Such an amount would be essential in maintaining the clinical loading dose and reducing the time interval between drug re-administration. Most likely, such an initial burst effect can be explained by the ability of the particles to quickly adhere to the particle surface and the subsequent adsorption. We also examined the pure drug and found a release amount of 98.9 % in 30 min. Obtained data was fitted to various kinetic models using the Sigma plot software version 9.01 (Peppas, Hixon and Crowell, Baker and Lonsdale, Higuchi and First order). Released data followed the Peppas model as demonstrated by the correlation coefficient ($R^2 = 0.986$). As such, the results demonstrate that the drug release followed a pattern of

initial swelling, following by diffuse dispersion. This pattern has also been demonstrated by other studies [24, 25].

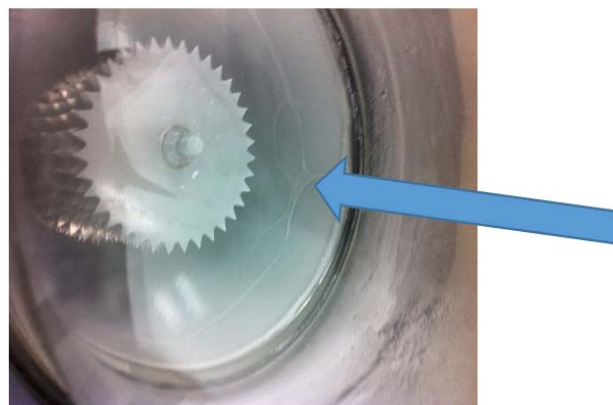


Figure 3: Drying chamber showing the particle adherence to the wall.

Polymer thermal analyses characterizes the chemical interactions between a drug and its polymer. The results demonstrated that the melting endotherm of drug was absent in thermogram indicates change in norfloxacin state from crystalline to amorphous as well its dispersion in molecular level in the nanoparticles (Figure 5). This demonstrates that the spray drying technology used to formulate nanoparticles creates a stable product. Data from stability studies signified that the external surface and encapsulation efficiency of prepared nanoparticles and did not show any significant variations from the original formulation. However, at 37 °C and with a RH of 75%, the moisture content lead to powder agglomeration. As such, product should be stored within a temperature range of 15-25 °C.

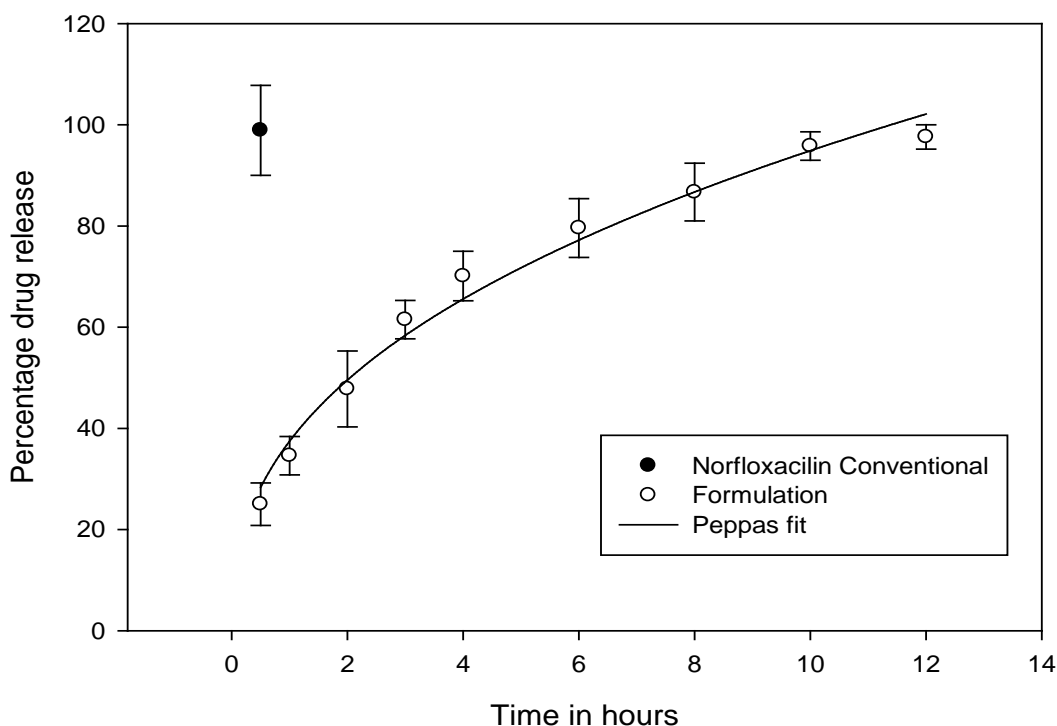


Figure 4: *In vitro* release profile of norfloxacin nanoparticles.

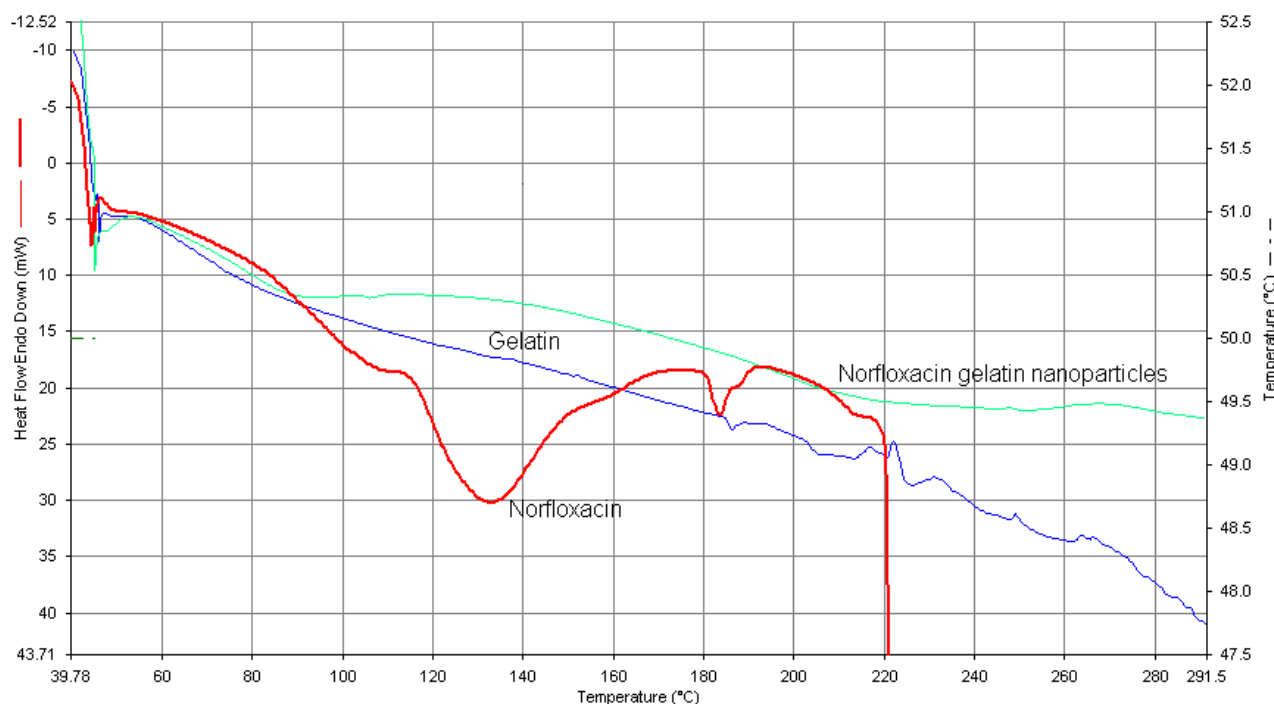


Figure 5: Incompatibility studies carried out using Differential Scanning Calorimetry for gelatin, norfloxacin and nanoparticles.

CONCLUSION

This work demonstrates the successful formulation of gelatin nanoparticles of norfloxacin using the patented Buchi Nano spray-drier B-90 equipment. As such, gelatin holds promise as a natural biodegradable and biocompatible polymer for use in targeted delivery systems. Formulated norfloxacin gelatin nanoparticles demonstrated a bimodal particle size distribution which accounted for the biphasic drug release pattern with a large bolus of release and a subsequent small release which together resulted in drug levels that could be maintained for up to 12 h. Furthermore, the surface of these gelatinized nanoparticles of norfloxacin were smooth with no cracks or holes present. Using differential scanning calorimetry, we found an absence of incompatibility, and the formulation was stable at a temperature range of 15–25 °C. The study provides a standard that can be hereby further modified in future studies for the formulation and preparation of gelatinized antimicrobials. Further work is needed to optimize production and test the drug delivery system in animal models.

Conflicts of interest

The author reports no conflicts of interest.

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