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# siRNA Loaded Nanoparticles: Serum Stability and In Vitro Release Studies

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## ABSTRACT

**Objective:** The objective of the present study is to examine the serum stability of siRNA loaded nanoparticles that were prepared with certain polymer and evaluate the influence of different pH values on the *in vitro* release of siRNA from these particles. **Methods:** Serum stability of siRNA loaded nanoparticles was studied by incubation in serum free medium and in presence of 10 %, and 25 % serum at 37ºC for 6 h. The unshielded fraction (liable to nucleases) of siRNA was measured by Fluoroskan microplate reader at $\lambda_{ex}$ = 485 nm, and $\lambda_{em}$ = 518 nm using fluorescence PicoGreen dye. The *in vitro* release of siRNA from nanoparticles, measured by electrophoresis, was studied in DMEM at two different pH values of 3.5 and 7.4. **Results:** The results of serum stability showed that 8.6 %, 24.4 %, and 23.5 % siRNA was unshielded from the nanoparticles in the presence of 0 %, 10 %, and 25 % serum, respectively. After 12 h, the released fraction of siRNA was 17.8 % and 95.2 % at pH 7.4 and pH 3.5, respectively. **Conclusion:** The obtained results revealed that the nanoparticles were considered as a reasonable delivery system that could protect siRNA from degradation by nucleases enzymes upon systemic injection and able to release the loaded siRNA in the acidic pH inside the required cells.

**Keywords:** *In vitro release; Nanoparticles; pH; Serum stability; siRNA.*

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## INTRODUCTION

The great capabilities of siRNA as an excellent gene silencer for treatment of many diseases such as AIDS, gene defects, cardiovascular, and neurodegenerative diseases[1-5], demonstrates its potential for siRNA-based therapeutics The first reported trial for siRNA delivery using nanoparticles as a targeted therapy in human through systemic injection was in 2010[6]. Recently, siRNA became more important in drug development and diseases treatment due to its elevated selectivity, significant effect, synthesis availability, and minor side effects.[5]

The cytoplasm of the cell is the main site of action of siRNA. Therefore, siRNA should be targeted to the cytosol of the specific cell type within the aimed tissue[7]. There are multiple barriers to the delivery of siRNA, which vary according to the route of administration and targeted tissue. In the systemic delivery of siRNA via intravenous injection, siRNA is distributed through blood circulation to the targeted organs and is eliminated form circulation in the same time. Inside the organ, it travels from the blood vessels to the interstitium tissues to be transported to the targeted cells. siRNA enters to the cell via encapsulation with the endosomes (endocytosis) and escapes to the cytoplasm and released from its loaded carrier showing the desired effect[8]. The reported plasma half-life of naked siRNA in literature is less than 10 min that is due to degradation with serum endonucleases and glomerular filtration.[9] These drawbacks were partially

solved with chemical modification of siRNA backbone or nano-carriers.[10]

High molecular weight, large size, and the negative charge of the phosphate backbone are the main limitations that reduce the ability of siRNA to pass through the anionic cell membrane via passive diffusion. Therefore, it was found that endocytosis is the major way for internalization of siRNA through the component of the cell membrane[11]. Following the endocytosis, the endocytosis vesicles fuse with the lysosome after passing the early and late endosomal stage, which result in releasing of the siRNA inside this vesicle and degradation of siRNA. The endosomal-lysosomal entrapment represents one of the most important limiting factors that lowering the transfection efficiency of non-viral siRNA nano-carriers.[12-14]

The objective of this study is to find a delivery system that can protect siRNA inside the circulation and shield it till reach endosomal vesicle to release siRNA load. The experimental work of the study deals with the stability of siRNA loaded nanoparticles in presence of different concentrations of serum (0-25 %). In addition to testing ability of the nanoparticles to protect siRNA from release at physiological pH of the blood (neutral), and allow for its release at the physiological pH of endosomal vesicle (acidic pH).

## MATERIALS AND METHODS

### Materials

Dulbecco's modified Eagle's medium DMEM (high glucose, pyruvate), OPTI-MEM reduced serum medium, fetal bovine serum (FBS), and phosphate buffered saline (PBS) were purchased from Gibco by life technologies, New York, USA. Silencer negative control siRNA was purchased from Ambion, California, USA. PicoGreen reagent was purchased from Thermo-Scientific, Foster city, California, USA.

### Stability of siRNA loaded nanoparticles in serum

The experiment was carried out to evaluate the stability of siRNA loaded nanoparticles in different concentrations of serum. siRNA loaded nanoparticles were incubated in presence of 0 %, 10 %, and 25 % serum at 37ºC for 6 h. After 6 h, the fraction of unshielded siRNA was determined by a Fluoroskan microplate reader (Thermo Fisher Scientific Inc., Waltham, MA) at $\lambda ex = 485$ nm and $\lambda em = 518$ nm as per manufactures' protocol using fluorescence intensities of PicoGreen dye. The fluorescence intensity of unshielded siRNA fractions was normalized to the fluorescence signal obtained upon mixing PicoGreen dye with siRNA (control) under the same conditions. The experiment was repeated in triplicates and the results were represented as mean ± SEM.

### In-vitro release study of siRNA from the loaded particles

The in vitro release profile of the siRNA from the loaded nanoparticles was studied in standard growth medium (DMEM, pH= 7.4) supplemented with serum, L-glutamine, sodium pyruvate, glucose and antibiotic mixtures with pH adjusted at 3.5. The samples were stirred at 100 rpm by using a magnetic stirrer at 37 ºC. At different time intervals 0, 1, 2, 3, 4, 5, 6, 8, 12 and 24 h, samples (20 µl) were withdrawn and kept at -20ºC till end of experiment. The samples were tested with gel electrophoresis and free siRNA was used as standard control. Before performing gel electrophoresis, the samples were kept till reach room temperature. The fraction of the siRNA displaced from nanoparticles was analyzed by conducting gel electrophoresis with 1 % w/v agarose gel stained with ethidium bromide, and siRNA bands were visualized under a UV camera. Moreover, the quantitative analysis was performed by using ImageJ software (NIH, Bethesda, MD), whereas the released siRNA was determined by comparing the shiny area of the free siRNA to the area of intended time point and expressed as percentage. Results represent the mean of three independent replicates ± SEM.

## RESULTS AND DISCUSSION

### Stability of siRNA loaded nanoparticles in serum

The normalized values of nanoparticles when compared to the free siRNA (control) showed that in absence of serum, the fraction of unshielded siRNA reaches 8.6 ± 1.8 %. This percentage increased to 24.4 ± 0.12 % and 23.5 ± 0.2 % when serum concentration increased to 10 % and 25 % respectively (Figure 1). These results have been shown even in presence of high percentage of serum. The fraction of unshielded siRNA (unprotected) not exceed 24 % and the remaining (76 %) of the dose still protected till 6 h after incubation with high concentration of serum. These results are compared with the reported half-life of unmodified siRNA in serum that usually ranges from few minutes to 60 minutes[15], it shows good stability for siRNA loaded with the selected nanoparticles than the normal unmodified one.

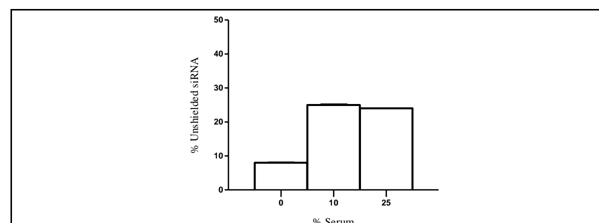


**Figure 1: Serum stability of siRNA loaded nanoparticles represented as % unshielded siRNA in presence of 0, 10, and 25% of serum.**

### *In vitro* release study of siRNA from nanoparticles

Figures 2 A and 2B show the images of 1% w/v agarose gel containing ethidium bromide showing the electrophoretic mobility of siRNA from loaded nanoparticles compared to free siRNA. The cumulative % release was calculated with addition of each time point % shiny area to the previous point.

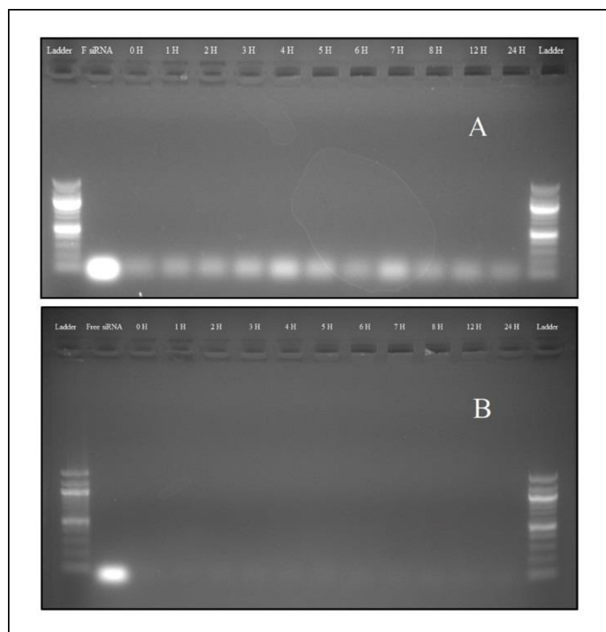


**Figure 2: Images of 1% w/v agarose gel containing ethidium bromide showing the electrophoretic mobility of siRNA from loaded nanoparticles compared to free siRNA at pH 3.5 (A) and pH 7.4 (B).**

Figure 3 shows the in vitro release profiles of siRNA from loaded nanoparticles at pH 3.5 and 7.4. The release profile of siRNA at pH 3.5 revealed that most of loaded siRNA was released within 8 h (86.5 ± 2.8 %), while exhibited nearly complete (95.2 ± 3.3 %) release within 12 h. On the other hand, release profile at pH 7.4 did not exceed 17.8 ± 0.64 % after 12 h and 18.6 ± 0.67 % within 24 h.

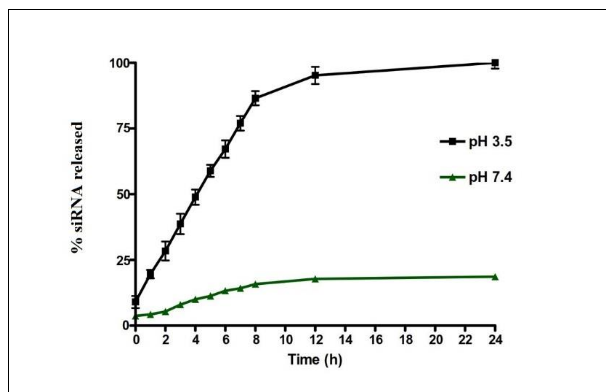


**Figure 3: In vitro % release profiles of siRNA from loaded nanoparticles at pH 7.4 and 3.5.**

These results indicated that after 24 h the fraction of siRNA released might be liable for degradation and lost in the circulation due to presence of serum and other blood components. These results are in good agreement with the previously discussed results of serum stability.

## CONCLUSION

The obtained results revealed that the nanoparticles were considered as a reasonable delivery system that could protect siRNA from degradation by nucleases enzymes upon systemic injection and able to release the loaded siRNA in the acidic pH inside the required cells.

### Conflict of Interest

The authors declare that they don't have any conflict of interest.

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