Drug Development and Industrial Pharmacy, 26(4), 459–463 (2000)

COMMUNICATION

Self-Assembled Carbohydrate-Stabilized Ceramic Nanoparticles for the Parenteral Delivery of Insulin

Anitha K. Cherian, A. C. Rana, and Sanjay K. Jain*

Pharmaceutics Laboratory, Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar, (M. P.) 470 003 India

ABSTRACT

The insulin-bearing aquasomes were fabricated by first preparing the nanosize calcium phosphate dihydrate core. The calcium phosphate dihydrate core was prepared by colloidal precipitation and sonication of disodium hydrogen phosphate solution and calcium chloride solution at low temperature. This core was coated with cellobiose, pyridoxal-5-phosphate, or trehalose under sonication and was further loaded with the drug at low temperature by a partial adsorption mechanism. The prepared systems were characterized for size, shape, size distribution, drug loading efficiency, and in vivo performance. The in vivo performance of the formulated aquasome was compared with standard porcine insulin solution, and better results were observed compared to insulin solution.

Key Words: *Aquasomes; Insulin; Nanoparticles; Self-assembly.*

INTRODUCTION

Many novel approaches to deliver a drug molecule at the site of action are being employed both experimentally and therapeutically to enhance the safety and effectiveness of pharmaceutical agents. The pharmacological molecules exhibit three activity-related spatial qualities: a unique three-dimensional conformation, a freedom of internal molecular rearrangement induced by intermolecular interaction, and a freedom of bulk movement. The loss of any of these activities by a pharmacologically active molecule yields one of many possible alternative conformations. This could be associated with the degradation, alteration, or loss of natural structural and functional properties (1).

The single greatest limiting force with insulin in the conversion of various delivery systems into useful clinical tools is the inevitable and destructive interaction

* To whom correspondence should be addressed.

between the drug carrier and the drug (2). In such a situation, carbohydrate-stabilized ceramic nanoparticles (aquasomes) are found to be promising. Disaccharides tend to stabilize biological macromolecules during desiccation due to creation of a quasi-aqueous environment that prevents dehydration-induced denaturation (1,3). Therefore, insulin-bearing aquasomes were developed to provide conformational stabilization, as well as a high degree of surface exposure for therapeutic response, and to protect against dehydration-induced denaturation of insulin.

EXPERIMENTAL

Materials

Porcine insulin was supplied from M/s M. J. Pharmaceutical (Baroda, India) as a gift sample. Trehalose and cellobiose (Himedia Laboratories [Pvt.] Ltd., Mumbai, India) and pyridoxal-5-phosphate (Central Drug House [Pvt.] Ltd., Mumbai, India) were used as received. Calcium phosphate was synthesized in the laboratory. All other chemicals were analytical grade and were used as received.

Preparation of Aquasomes

The calcium phosphate dihydrate cores were prepared by a method reported by Kossovsky (4). A 0.75 M solution of Na_2HPO_4 was slowly added to the 0.25 M solution of $CaCl_2$ under sonication and was sonicated for 2 hr at 4°C. The precipitate (calcium phosphate) was separated by centrifugation at 15,000 rpm for 1 hr and then washed five times with 50 ml of distilled water to remove sodium chloride formed during the reaction. The precipitate was resuspended in the distilled water and passed through a 0.2-μm Millipore filter to collect particles less than 0.2 μm. These core particles were coated with polyhydroxy oligomer under sonication and lyophilization. The process variables (like core-to-coat ratio, applied power of sonicator, and sonication time) were optimized to get small (100–200 nm) spherical, discrete, coated nanoparticles (Table 1). Finally, the drug was loaded to these coated nanoparticles by partial adsorption of a 0.1% solu-

Table 1

Effect of Various Process Variables on the Formation of Aquasome (Without Drug)

Variables	Results	Size (nm)
Core:coat[a]		
1:1	Irregular	164
1:2	Irregular	178
1:3	Almost spherical	184
1:4[b]	Spherical structures	196
1:5	Spherical structures	196
Sonicator power (W)[c]		
5	More large discrete particles	228
10	Comparatively large discrete particles	202
15[b]	Small spherical discrete particles	184
20	Small spherical discrete particles	174
Sonication time (min)[d]		
20	Large irregular particles	234
30	Large discrete particles	220
45	Moderate discrete particles	202
60[b]	Small spherical discrete particles	180
90	Small aggregates	104

[a] Sonication time 60 min and power of sonication 15 W.
[b] Selected variable.
[c] Core:coat 1:4 and sonication time 60 min.
[d] Core:coat 1:4 and power of sonicator 15 W.

tion of insulin in saline phosphate buffer (SPB), pH 7.4, for 24 hr. All the operations were carried out at a temperature below 4°C.

Characterization

Size and Size Distribution

The average size and size distribution of aquasomes were determined by transmission electron microscopy after negative staining with 1% phosphotungstic acid (Fig. 1).

Drug Payload

Drug loading was determined using a method reported by Loukas and Gregoriadis (5). The various plain aquasome formulations (without drug coating) were incubated with the known concentration of drug for 24 hr at 4°C. The supernatant was separated after centrifugation at 15,000 rpm for 1 hr below 4°C in a refrigerated centrifuge (Sico Eltek refrigerated centrifuge R C 4100, Lucknow, India). The drug remaining in the supernatant liquid after loading was estimated by measuring absorbance at 276 nm using a spectrophotometer (Shimadzu ultraviolet-visible [UV-Vis] spectrophotometer 1601, Tokyo, Japan).

In Vivo Performance Study

The in vivo evaluation of various aquasome formulation(s) of insulin were performed on albino rabbits of either sex weighing 2–2.5 kg (6). Animals were divided into five groups (A–E) of 3 animals each. The animals of group A were treated as controls. Animals of group B were treated as the standard group and received porcine insulin subcutaneously (s.c.) at a dose of 2.5 U/kg, whereas animals of groups C, D, and E received an equivalent dose of insulin subcutaneously from the various formulation(s), Aq.Ce., Aq.Py., and Aq.Tr., respectively. Animals fasted overnight to assess the steady-state blood glucose levels. Blood samples were collected from a lateral marginal vein by venipuncture. After collecting blood samples at zero hour, the standard insulin or the formulation(s) were administered to the respective animals. After the administration of the drug, blood samples were withdrawn periodically (every hour) for 24 hr to assess the blood glucose level. Blood glucose concentrations in the samples were determined using a chemistry analyzer (RA 150 Aimes, Baroda, India).

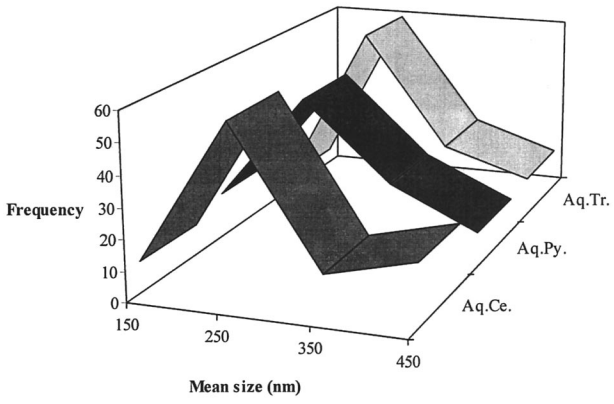

Figure 1. Size distribution of various drug-bearing aquasomes.

RESULTS AND DISCUSSION

The aquasomes were prepared using the method reported by Kossovsky (4). The nanocrystalline calcium phosphate dihydrate ceramic cores self-assemble during the reaction process under sonication. The sonication helps the self-assembling of the crystalline calcium phosphate particles by increasing the surface free energy. These nanoparticles were further coated with the thin film of polyhydroxy oligomer by sonicating with the polyhydroxy oligomers (Trehalose, cellobiose, or pyridoxol-5-phosphate coating materials). The polyhydroxy oligomer stabilized the core through ionic, noncovalent, and extropic forces. These stabilized carriers (aquasomes) serve as nondenaturing solid carriers for the delivery of the drug candidate.

The effects of various process variables (i.e., core-to-coat ratio, applied power of sonicator, and sonication time) were studied. It is noted that, on increasing the core:coat ratio from 1:1 to 1:5, the size of the aquasome increased from 164 nm to 196 nm. The size of the coated particles increased up to 1:4 core-to-coat ratio, and then no increase in size was observed. This would be due to the saturation of the free surfaces of the core with the coating material. But, on increasing the sonication time from 20 to 60 min, the size and shape of the aquasomes changed from irregular large particles (234 nm) to small uniform particles (180 nm); at 90 min, the aggregates of small particles were observed. Similarly, the size decreased from 228 nm to 174 nm on increasing power of the sonicator (5 to 20 W); at 20 W, they showed a tendency to aggregate. The aggregation of the small particles was observed after 15 W sonication power and after 60 min due to an increase in free surface energy, which in-

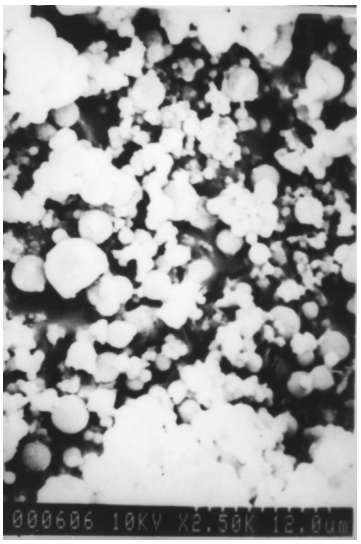

Figure 2. Electron microphotograph of aquasome prepared with pyridoxal-5-phosphate coating (15,000×).

creases the tendency of small particles to aggregate. Therefore, a 1:4 core-to-coat ratio, 60 min sonication time, and 15 W power of sonicator were selected to get spherical, coated, discrete particles (aquasomes).

Finally, the drug was allowed to adsorb on the coated nanoparticulate surfaces by a partial adsorption technique. It is reported that polyhydroxy oligomer film prevents ''soft'' drug from changing shape and being damaged when surface bound. The polyhydroxy oligomers create a glossy film and act as dehydroprotectants (7). These surface-modified nanoparticles provide conformational stabilization, as well as a high degree of surface exposure, to proteins (6).

The drug-loaded aquasomes were characterized for size and shape. The results indicate that they are spherical in shape, having a size below 300 nm (Fig. 1 and 2). Drug loading of 53.68% ± 4.64% was observed with Aq.Ce. (size 281 nm), whereas Aq.Py. (size 293 nm) and Aq.Tr. (size 281 nm) showed drug loading of 59.74% ± 3.65% and 48.98% ± 3.96%, respectively. The size of aquasomes increased about 300 nm due to adsorption of drug molecules on the surface of cores coated with polyhydroxy oligomer. The increased drug payload with Aq.Py. was observed due to the increase in size.

The in vivo performances of drug-bearing aquasomes and the standard plain insulin solution were assessed by periodical measurement of blood glucose level for 24 hr in diabetes-induced albino rabbits after a subcutaneous dose of 2.5 U/kg, and the percentage blood glucose reduction was calculated (Table 2). The initial or zero-hour blood glucose level used was 100%.

The porcine insulin solution showed a maximum blood glucose reduction (74.92% ± 0.88%) after 1 hr, which then gradually increased to a normal blood glucose level, while formulation(s) Aq.Ce, Aq.Tr., and Aq.Py. exhibited maximum blood glucose reductions of 44.48% ± 0.43%, 80.70% ± 1.95%, and 91.40% ± 0.90%, respectively, within 2 to 3 hr. The prolonged reduction in blood glucose was observed with all formulations except Aq.Ce. The percentages of blood glucose reduction and duration of action for Aq.Tr. and Aq.Py. are significantly higher ($p < .02$) compared to porcine

Table 2

Percentage Blood Glucose Reduction After Subcutaneous Administration of Plain Insulin and Various Aquasome Formulation(s) to Albino Rabbits

	Blood Glucose Reduction[a] (%)			
Time (hr)	Pl.	Aq.Ce.	Aq.Py.	Aq.Tr.
0	0	0	0	0
1	74.92 ± 0.88	40.75 ± 0.36	75.57 ± 0.20	70.19 ± 1.40
2	61.85 ± 0.48	44.48 ± 0.43	90.6 ± 0.88	80.70 ± 1.95
3	50.98 ± 0.77	30.18 ± 0.35	91.40 ± 0.90	68.98 ± 1.96
4	26.56 ± 0.33	23.88 ± 0.09	84.10 ± 0.58	59.09 ± 2.23
5	11.76 ± 0.24	5.65 ± 0.40	72.49 ± 0.58	50.73 ± 2.14
6[b]	3.06 ± 0.88	2.54 ± 0.27	64.60 ± 0.58	42.24 ± 3.58
24	—	—	13.93 ± 0.56	—

[a] Each value represents the mean ± standard deviation of three determinations.
[b] Food was given to rabbits after 6 hr.

insulin solution, which proves the efficacy of the formulation.

Cellobiose, trehalose, and pyridoxal-5-phosphate protect the drug molecule against dehydration; however, trehalose and pyridoxal-5-phosphate are significantly more effective, which was also reported by Green and Angell (8). Moreover, Aq.Py. reduces blood glucose more effectively compared to Aq.Ce. and Aq.Tr., which could be due to the high degree of molecular preservation by virtue of a significant degree of retained biological activity (6). The polyhydroxy oligomers act like water since they are enriched with hydroxyl groups and give a waterlike atmosphere. They may even replace the water around the polar residues in proteins, thereby maintaining their integrity in the absence of water. The prolonged activity obtained may be by the slow release of the drug moiety from the drug carrier and be due to the intact structure without denaturation or dehydration during delivery and storage.

It is concluded that aquasomes were found to be promising for protection of the spatial qualities of the peptide drug for exhibiting better therapeutic effect. However, extensive pharmacokinetic, clinical, and toxicological studies are required to launch the formulation in the market.

ACKNOWLEDGMENTS

We thank the University Grants Commission, Ministry of Human Resources Development, Government of India, for the financial support; M/s M. J. Pharmaceuticals, Baroda, India, for the gift sample of porcine insulin; RSIC, Nagpur, India, for transmission electron micrography; and Dr. Harisingh Gour Vishwavidyalaya, Head, Department of Pharmaceutical Sciences, Sagar, India, for making available the facilities to carry out the work.

REFERENCES

1. N. Kossovsky, Biotechnology, 11, 1534–1536 (1993).
2. C. Damge, C. Michael, M. Aprahamian, and P. Couvreur, Diabetologia, 29, 531A (1986).
3. N. Kossovsky, A. Gelman, E. E. Sponsler, H. J. Hnatyzyn, S. Rajguru, M. Torres, J. Crowder, J. Zemanovich, A. Chung, and R. Shah, Biomaterials, 15, 1201–1207 (1994).
4. N. Kossovsky, *Artificial Self-Assembling Systems for Gene Delivery*, 1996, chap. 15, pp. 152–168.
5. Y. L. Loukas and G. Gregoriadis, Proc. Int. Symp. Controlled Release Bioact. Mater., 24, 404, 204 (1997).
6. N. Kossovsky, A. Gelman, S. Rajguru, R. Nguyen, E. Sponsler, H. J. Hnatyszyn, K. Chung, A. Chung, M. Torres, J. Zemanovich, J. Crowder, P. Barnajian, K. Ly, J. Philipose, D. Ammons, S. Anderson, C. Goodwin, P. Soliemanzadeh, G. Yao, and K. Wei, J. Controlled Release, 39, 383–388 (1996).
7. N. Kossovsky, D. Millett, E. D. Sponsler, and H. J. Hnatyszyn, Biotechnology, 11, 1535–1536 (1993).
8. J. L. Green and C. A. Angell, J. Phys. Chem., 93, 2880–2882 (1989).