Preparation of Cyanoacrylate Nanoparticles Using Monosaccharides or Disaccharides

Shoichi SHIROTAKE,* Yu'usuke TOMITA, Shinji YOSHIZAKI, and Kenji OKUDA

Department of Pharmaco-Therapeutics, Graduate School of Medicine, Yokohama City University; 3–9 Fuku'ura, Kanazawa-ku, Yokohama 236–0004, Japan.

Received May 14, 2007; November 5, 2007; published online November 7, 2007

By using biomaterials, including monosaccharides and disaccharides, as polymeric stabilizing agents, the cyanoacrylate nanoparticles was prepared respectively, with particles diameter of approximately 200 nm or 300 nm. The new method was applied to load/encapsulate ampicillin (ABPC) and pDNA into nanoparticles. Loading efficiency of ABPC was increased compared to the existing method in which dextran is used as a stabilizer. The pDNA encapusulation rate was 68.7%, by using glucose.

Key words cyanoacrylate; nanoparticle; saccharide; encapsulation

The side effects of drugs can be a serious issue during drug therapy. Thus, reducing the toxicity associated with drugs is a major object of pharmaceutics.

In order to develop a safe drug formulation, Drug Delivery Systems (DDS) have been studied. Recently, there have been extensive investigations of DDS based on nanotechnology.[1—5] Here, we have prepared drug encapsulated nanoparticles of different particle size in order to properly using drug potency.

Nanoparticles are broadly divided into lipoic- and acrylic-types according to the polymer material used in their fabrication. Significant research effort in Japan has focused on the lipoic-type polymers.[4,5] Lipoic-type nanoparticles possess several attributes, including ease of preparation and a high rate of intracellular drug delivery. However, these particles tend to have an unstable size distribution and can readily incorporate impurities during sample preparation. Therefore, in order to synthesize nanoparticles of homogenous particle size we have investigated the use of *n*-butyl cyanoacrylate (NBCA: Histoacryl®), which is currently utilized as a surgical adhesive. Because polymerization conditions determine the physicochemical properties of nanoparticles, changing these conditions results in a diverse range of particle size.[6—8] The type and concentration of polymeric stabilizing agent greatly impacts on the properties of drug encapsulated nanoparticles during the preparation of poly-alkyl cyanoacrylate nanoparticles.

In the present study, we have developed a new method for preparing nanoparticles in which mono- and disaccharides were used for investigating polymerization conditions of poly (butyl cyanoacrylate) nanoparticles. Furthermore, we applied this new method to the preparation of ampicillin (ABPC)- or pDNA-encapsulated nanoparticles.

Experimental

n-Butyl 2-cyanoacrylate (nBCA: Histoacryl®) was generously provided by B/BRAUN Aesculap AG & Co. (Tuttlingen, Germany). Dextran-10000 (Dex-10), Dextran-70000 (Dex-70), Polysorbate (Tween-20) and Ampicillin (ABPC) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Fructose, glucose, mannose, ribose, lactose, maltose, sucrose, trehalose, 1 N HCl solution and 0.1 N NaOH were purchased from Wako (Tokyo, Japan). Plasmid DNA (pCMVrev/env) was prepared according to the previously described method.[9]

One gram of fructose, glucose, mannose, ribose, lactose, maltose, sucrose or trehalose was dissolved to 20 ml of 0.001 N HCl and then NBCA (1%) was added under mechanical stirring. After 2 h of continuous stirring, the reaction mixture was neutralized with 0.1 N NaOH followed by 0.5 h stirring to obtain a colloidal suspension. The colloidal suspension of nanoparticles was then filtered through a Millex filter unit (pore size: 5 μm). Nanoparticles were rinsed five times with distilled water using the centrifugal filter devices (CENTRIPREP® CY-10, Millipore Corp., Bedford, MA, U.S.A.) before evaluating the physicochemical properties. ABPC-encapsulated nanoparticles were synthesized in 0.01 N HCl solution of ABPC (0.2%), glucose (sugar; 5%) and NBCA (1%). In addition, an ABPC concentration of the initial filtrate was obtained with the optical density method (λ_{max} 254 nm) and defined as released ABPC amount that was not encapsulated in nanoparticles. The ABPC loading rate of ABPC-encapsulated nanoparticles was calculated from encapsulated amount divided by additive amount: (encapsulated amount=additive ABPC−filtrated ABPC). Plasmid DNA-encapsulation was carried out in a solution of pDNA (0.1%), glucose (sugar; 5%) and NBCA (1%). Plasmid DNA concentration of the initial filtrate was obtained with the optical density at 260 nm and defined as the pDNA amount not loaded into nanoparticles. Preparation of nanoparticles using dextran or polysorbate was performed according to the methods[7,8] by Douglas *et al.*

After filtering the colloidal suspensions using Nuclepore Track-Etch Membrane of pore size 0.1 μm (Whatman, Clifton, NJ, U.S.A.), morphological analyses of nanoparticles attached on the membrane were performed by examining the particles under a scanning electron microscopy (SEM; S-800, Hitachi Corp., Tokyo, Japan). The particle size was measured by using both SEM and dynamic light scattering (DLS) analysis (He–Ne laser; Zetasizer Nano; Malvern Instruments Ltd., Malvern, U.K.). The zeta potential was determined by Zetasizer Nano (Malvern Instruments Ltd., Malvern, U.K.).

Results and Discussion

Cyanoacrylate nanoparticles were obtained as stable solutions within a relatively short time (2 h) by adding nBCA at a final concentration of 1 to 5% solution of sugar (mono- or disaccharide) in 0.001 N HCl at room temperature. As shown in Table 1, nanoparticles with approximate diameters of 200 and 300 nm were obtained when using monosaccharides and disaccharides as polymerization stabilizing agents, respectively. However, approximate average particle sizes of 159 and 150 nm were obtained using Dex-10 or Dex-70, respectively. The smallest particle sizes (57.1±21.7 nm; mean± S.D.) were obtained using Tween 20. The size of nanoparticles prepared using monosaccharides and disaccharides as stabilizing agents were larger than those using dextran and polysorbates,[7,8] although the underlying mechanism for

Table 1. Nanoparticle Size and Zeta Potential

Saccharides	Size (mean±S.D.) (nm)	Zeta potential (mV)
Fructose	201±30.9	−51.7
Galactose	203±23.9	−53.0
Glucose	206±29.0	−51.9
Mannose	191±21.7	−57.9
Ribose	175±19.9	−63.8
Lactose	299±29.8	−57.8
Maltose	300±42.0	−56.2
Sucrose	295±23.3	−52.7
Trehalose	322±34.7	1.06
Dextran-10000	159±30.4	−28.6
Dextran-70000	151±27.6	−12.8

* To whom correspondence should be addressed. e-mail: shirophd@med.yokohama-cu.ac.jp

this observation is unclear in the present report. The zeta potentials of the obtained nanoparticles were determined to be negative except for those prepared using trehalose. Zeta potentials for the nanoparticles prepared using monosaccharides and disaccharides were considerably larger than those prepared using dextran, suggesting more porous particles.[8,11] The size and zeta potential of nanoparticles were almost invariable for 1 month, as those prepared using glucose [initial: 206±29.0 nm (mean±S.D.) and −51.9 mV; one month later: 204±28.6 nm and −51.9 mV]. The colloidal suspensions were stable for a prolonged period of time, suggesting that the incorporated saccharides are likely to contribute to long-term stability. The size and zeta potential of nanoparticles prepared using glucose for encapsulating ABPC were 217±29.1 nm and −55.0 mV, respectively. Those for encapsulating pDNA, which has a large molecular weight, were 395±40.9 nm and −69.5 mV, respectively. These values are considerably greater than those obtained for empty nanoparticles. SEM showed the morphological appearance of the particles to be that of an ultra fine sphere (Fig. 1). Particle sizes obtained by SEM corresponded to the values determined in the DLS analysis. The encapsulating/loading efficiency of ABPC into nanoparticles prepared using glucose was 24.8%, which was significantly higher than nanoparticles[10] prepared using Dextran-70 (16.3%). The pDNA encapusulation rate was 68.7%.

We have shown that monosaccharides and disaccharides as well as dextran and polysorbate can contribute to the preparation of cyanoacrylate nanoparticles. With the new method, it can be efficiently encapusulated into nanoparticles not only drug but also high molecular weight molecules such as plasmid DNA.

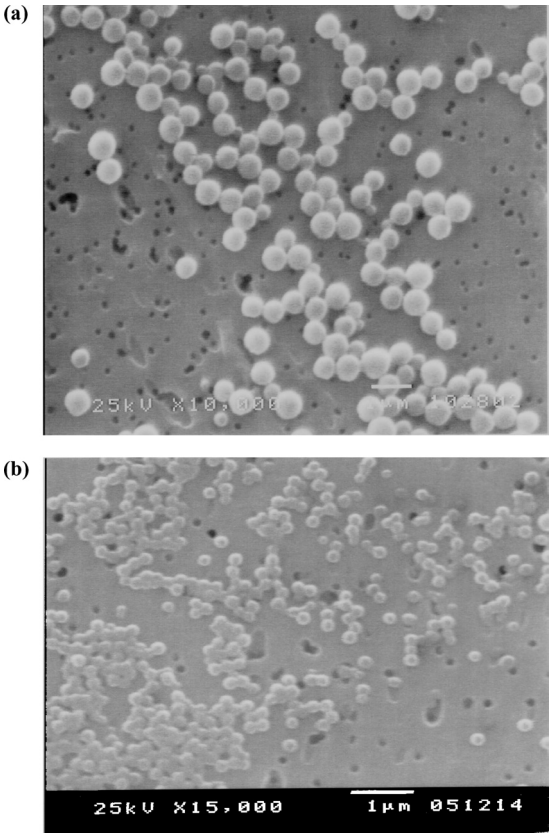

Fig. 1. Morphological Appearance of pDNA-Encapsulated Nanoparticles and ABPC-Encapsulated Nanoparticles (SEM)

(a) pDNA-encapsulated nanoparticles, ×10000. (b) ABPC-encapsulated nanoparticles, ×15000.

References

1) Yokoyama M., *Drug Delivery System*, **14**, 449—457 (1999).
2) Ishida O., Maruyama K., Sasaki K., Iwatsuru M., *Int. J. Pharm.*, **190**, 49—56 (1999).
3) Ravi Kumar M. N. V., *J. Pharm. Pharmaceut. Sci.*, **3**, 234—258 (2000).
4) Sezaki H., Hashida M., *CRC Crit. Rev. Ther. Drug Carrier Syst.*, **1**, 1—18 (1985).
5) Muller R. H., Mader K., Gohla S., *Eur. J. Pharm. Biopharm.*, **50**, 16—177 (2000).
6) Horn D., Rieger J., *Angew. Chem. Int. Ed.*, **40**, 4330—4361 (2001).
7) Douglas S. J., Illum L., Davis S. S., *J. Colloid Interface Sci.*, **103**, 154—163 (1985).
8) Alonso M. J., Sanchez A., Torres D., Seijo B., Vila-Jato J. T., *J. Microencapsulation*, **7**, 517—526 (1990).
9) Jonai N., Okuda K., Kojima Y., Toda Y., Hamajima K., Ohba K., Klinman D., Xin K. Q., *J. Gene Med.*, **5**, 609—617 (2003).
10) Fontana G., Pitarresi G., Tomarchio V., Carlisi B., Biagio P. L. S., *Biomaterials*, **19**, 1009—1017 (1998).
11) Reddy L. H., Murthy R. R., *Acta Pharm.*, **54**, 103—118 (2004).