Department of Pharmaceutics, Bombay College of Pharmacy, Kalina, Santacruz (East), Mumbai, India

Organic solvent-free approach to single step fabrication of Eudragit nanoparticles using Labrasol

A. A. DATE, A. JAIN, P. KHACHANE, M. S. NAGARSENKER

Received April 25, 2010, accepted March 7, 2010

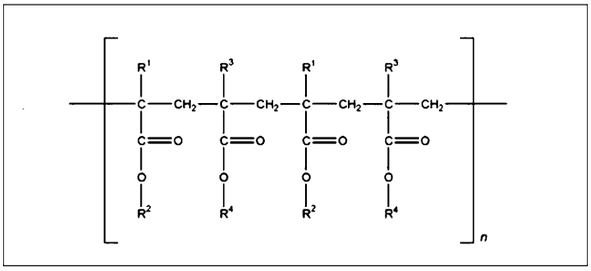
Mangal S. Nagarsenker, Department of Pharmaceutics, Bombay College of Pharmacy, Kalina, Santacruz (East), Mumbai-400098, India
mangal_nag511@yahoo.co.in

Pharmazie 65: 733–736 (2010) *doi: 10.1691/ph.2010.0063*

The present investigation describes organic volatile solvent-free approach for the single step fabrication of Eudragit nanoparticles. The solubility of various grades of Eudragit viz. Eudragit L100-55, Eudragit L100, Eudragit S100, Eudragit EPO, Eudragit RSPO and Eudragit RLPO in Labrasol® (Caprylocaproyl macrogol-8 glycerides) was determined. We observed that Labrasol® has the ability to solubilize various cationic Eudragits such as Eudragit RSPO, Eudragit RLPO and Eudragit EPO at a concentration as high as 200 mg/ml. We also evaluated the ability of Labrasol® to act as a nanoparticle stabilizer due to its amphiphilic nature and high HLB of 14. It was observed that Eudragit EPO, Eudragit RSPO and Eudragit RLPO nanoparticles of size 110–150 nm could be easily fabricated using Labrasol as a solubilizer and nanoparticle stabilizer. Transmission electron microscopy was carried out to confirm the size and morphology of the nanoparticles. We could encapsulate hydrophobic drugs such as repaglinide and triclosan in the Eudragit RLPO nanoparticles as Labrasol also had the ability to solubilize these hydrophobic drugs. The ability of Eudragit RSPO and RLPO nanoparticles to condense plasmid DNA was also established. This is the first report that demonstrates the polymer solubilizing potential of Labrasol.

1. Introduction

Eudragit represents a brand name for a range of methacrylate copolymers that are commercially available for various pharmaceutical applications (Fig. 1). Different types of Eudragit are available for diverse oral applications such as gastric release (Eudragit E100), enteric release (Eudragit L100-55), colonic release (Eudragit L100, Eudragit S100) and controlled release (Eudragit RLPO and Eudragit RSPO) (Varshosaz et al. 2006; Dong et al. 2007; Asghar et al. 2009; Yen et al. 2009). Certain types of Eudragit can also be used as a film former in dermal and transdermal applications (Lieb et al. 2002; Kanikkannan et al. 2004). There are numerous reports on the utility of various Eudragit copolymers for the applications mentioned above. Moreover, the US Food and Drug Administration has approved these polymers for such applications.



For *Eudragit E*: R^1. $R^3 = CH_3$ $R^2 = CH_2CH_2N(CH_3)_2$ $R^4 = CH_3$. C_4H_9

For *Eudragit L* and *Eudragit S*: R^1. $R^3 = CH_3$ $R^2 = H$ $R^4 = CH_3$

For *Eudragit RL* and *Eudragit RS*:

$R^1 = H$. CH_3 $R^2 = CH_3$. C_2H_5 $R^3 = CH_3$ $R^4 = CH_2CH_2N(CH_3)_3^+Cl^{-a}$

Fig. 1: Structure of various Eudragit copolymers

In recent years, attention has also been focused on the fabrication of Eudragit nanoparticles for various applications. Researchers have demonstrated that Eudragit nanoparticles (Eudragit E100, Eudragit RSPO, Eudragit RLPO or Eudragit L100-55) can be used to improve the bioavailability of various hydrophobic drugs such as ciclosporine A, aciclovir and naringenin (Yang et al. 2009; Yen et al. 2009; Elshafeey et al. 2010). Researchers have also demonstrated that Eudragit nanoparticles can be used for improving the therapeutic efficacy of various hydrophobic drugs in ocular and transmucosal delivery (Bucolo et al. 2002; Lopedota et al. 2009). Thus, Eudragit nanoparticles show good potential for improving the therapeutic performance of drugs by various routes of administration.

Although considerable research has been done on exploring applications of Eudragit nanoparticles in drug delivery, few efforts have focused on identifying greener approaches to the fabrication of Eudragit nanoparticles. Until now, researchers have routinely used various organic solvents such as acetone, ethyl acetate, dichloromethane, chloroform and ethanol (Pinto Reis et al. 2006) to fabricate polymeric nanoparticles including those with Eudragit. The organic solvents can be removed by vacuum evaporation using a rotary evaporator. Vacuum evaporation seems easy on a lab scale, but it presents several difficulties in scale up reproducibility on a large scale. This step also dramatically increases the manufacturing cost of the

nanocarriers. Furthermore, organic solvents always pose several environmental concerns. Thus, the use of organic solvents raises concerns with respect to scale up and biocompatibility. Until now, there have been no reports on the organic solvent-free fabrication of Eudragit nanoparticles. In the present investigation, we report organic solvent-free fabrication of various Eudragit nanoparticles by using a pharmaceutically acceptable amphiphilic stabilizer, Labrasol.

Labrasol (caprylocaproyl macrogol-8 glycerides) is an amphiphilic stabilizer having very good acceptability for oral and dermal delivery ($LD_{50} > 1$ g/kg in rats according to the manufacturer's information). Several investigations have established the utility of Labrasol in improving oral and transdermal delivery of various drugs (Zhao et al. 2006). It has been demonstrated that Labrasol has ability to inhibit P-gp efflux and to increase Caco-2 permeability (Sha et al. 2005). Labrasol has been widely used as a stabilizer for the fabrication of oral and topical microemulsions (Djekic and Primorac 2008). However, so far there have been no reports on the utility of Labrasol in the fabrication of Eudragit nanoparticles. In our preliminary studies using Labrasol as a polymeric nanoparticle stabilizer, we observed that Labrasol had the ability to solubilize Eudragit RSPO. This was an unexpected observation and it encouraged us to evaluate the ability of Labrasol to solubilize various types of Eudragit.

2. Investigations and results

2.1. Solubility of various Eudragits in Labrasol and fabrication of Eudragit nanoparticles

Interestingly, we observed that Labrasol could solubilize various polymers such as Eudragit RSPO, Eudragit RLPO and Eudragit EPO at a concentration as high as 200 mg/ml. Until now, nothing has been reported about the ability of Labrasol to solubilize these polymers. Other types of Eudragit such as L100-55, L100 and S100 were not soluble in Labrasol. Interestingly, Eudragit RSPO, RLPO and EPO nanoparticles could be successfully fabricated using Labrasol. The particle sizes of various Eudragit nanoparticles are given in Table 1.

Table 1: Mean particle size and polydispersity index of Eudragit nanoparticles fabricated using Labrasol (n = 3)

Nanoparticles	Particle size	Polydispersity index
Eudragit RSPO	125.4 ± 4.25	0.578 ± 0.137
Eudragit RLPO	92.4 ± 1.30	0.372 ± 0.060
Eudragit EPO	141.9 ± 2.85	0.147 ± 0.017

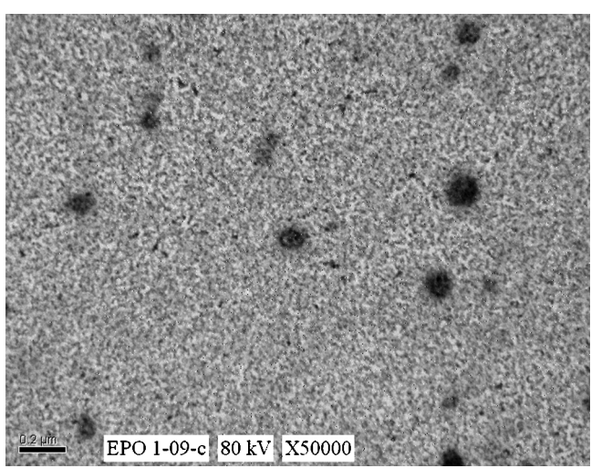


Fig. 2: TEM image of EPO nanoparticles

Table 2: Mean particle size and polydispersity index of drug loaded Eudragit RLPO nanoparticles and encapsulation efficiency (n = 3)

Drug	Particle size (nm)	Polydispersity index	Encapsulation efficiency
Repaglinide	$102.7. \pm 4.3$	0.578 ± 0.14	98.2 ± 0.6
Triclosan	232.4 ± 6.5	0.26 ± 0.070	88.2 ± 2.1

2.2. Characterization of Eudragit nanoparticles

The Eudragit nanoparticles were characterized with respect to zeta potential. The zeta potential values obtained for the Eudragit RSPO, Eudragit RLPO and EPO nanoparticles were $+39.6 \pm 1.6$ mV, $+42.3 \pm 2.1$ mV and 33.2 ± 1.5 mV respectively. It is well known that Eudrgit RSPO, RLPO and EPO have aminomethacrylate side chains. Hence, it would be expected that these nanoparticles would have positive zeta potential values. A representative TEM of Eudragit EPO nanoparticles is shown in Fig. 2. It is evident that these nanoparticles are spherical in form and the particle size obtained by TEM is in accordance with that obtained by photon correlation spectroscopy.

2.3. Fabrication of drug loaded Eudragit nanoparticles

Eudragit RLPO was selected for encapsulation of hydrophobic drugs. Two hydrophobic drugs, repaglinide and triclosan, were encapsulated in Eudragit RLPO nanoparticles. The results of particle size and encapsulation efficiency are shown in Table 2. The repaglinide loading obtained in nanoparticles was 1% w/w of the polymer whereas in the case of triclosan, a 1% w/w loading could be obtained.

2.4. Ability of Eudragit RSPO and RLPO nanoparticles to condense plasmid DNA (pDNA)

It has been demonstrated that Eudragit RSPO and RLPO have potential in ocular delivery. Also, they have been used as a matrix for transdermal patches. Furthermore, they are positively charged. Hence, Eudragit RSPO and RLPO nanoparticles could have potential for the ocular and dermal delivery of plasmid DNA. The results of the pDNA condensation study are shown in Fig. 3. It is evident from the figure that Eudragit RSPO and RLPO nanoparticle could both condense pDNA at a pDNA:nanoparticle ratio of 1:10 and 1:15. The ratios 1:1 and 1:5 revealed partial complexation of pDNA as the electrophoretic pattern differs considerably from that of naked pDNA. The condensation ability of RLPO nanoparticles appeared to be greater compared with that of RSPO nanoparticles, as is evident from the figure.

3. Discussion

In the present investigation, we demonstrated for the first time that Eudragit nanoparticles could be fabricated without using commonly used volatile organic solvents. We observed that Labrasol (caprylocaproyl macrogol-8 glycerides) can act as a solvent for cationic forms of Eudragit such as Eudragit EPO, Eudragit RLPO and Eudragit RSPO.

Labrasol is commonly included in various lipid based formulations such as microemulsions due to its ability to act as a solubilizer and permeability/bioavailability enhancer for various hydrophobic drugs. Due to its high HLB value of 14, Labrasol

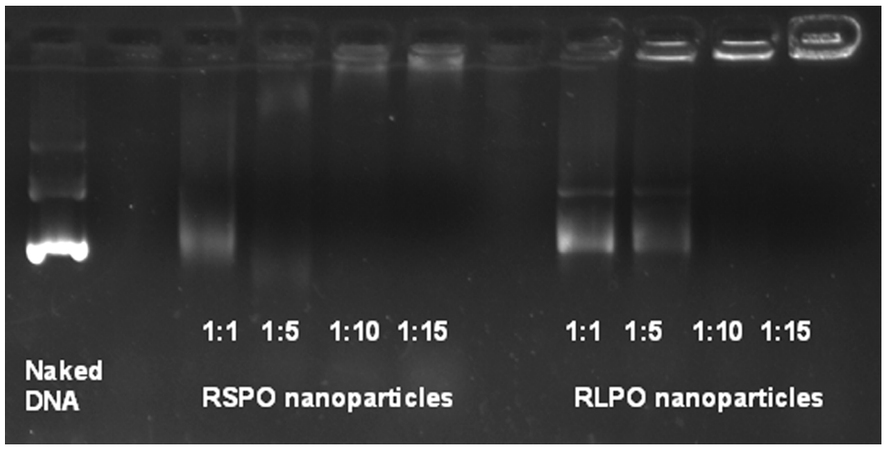


Fig. 3: pDNA condensation ability of RLPO and RSPO nanoparticles

can also act as a stabilizer. As we believed that Labrasol can act as a polymer solubilizer and nanoparticle stabilizer, we did not employ any surfactant in the aqueous phase. Interestingly, our results indicate that Eudragit nanoparticles could be successfully fabricated, confirming the dual role of Labrasol as solubilizer and stabilizer for nanoparticles. The final concentration of the Labrasol in the nanoparticle dispersion was 5% v/v. This concentration of Labrasol is highly acceptable for various delivery routes, such as oral, dermal, nasal and ocular. Since Labrasol is a biodegradable solvent with safe degradation products (physiological short chain lipids), there are no issues associated with the use of Labrasol. Hence, Labrasol need not be removed from the nanoparticle dispersion. In fact, the presence of Labrasol may further enhance the performance of the nanoparticles due to its well established permeation enhancing property. Thus, Eudragit nanoparticles can be successfully fabricated using a biocompatible solvent.

It is noteworthy that the other routinely used stabilizers such as Tween 80 or Cremophor EL have no such ability to solubilize various polymers (data not shown). It is also noteworthy that only those Eudragit copolymers which have amino groups in their backbone were soluble in Labrasol. Hence, to understand the reason for the solubilizing potential of Labrasol, we determined its pH It was observed that Labrasol exhibits an acidic pH due to the presence of a small amount of free fatty acids such as caprylic acid. We observed that short chain fatty acids such as caprylic acid can solubilize cationic Eudragit (data not shown). However, since the amount of fatty acids present in Labrasol is very small, this is not the only reason for its solubilizing potential. We shall perform further investigations of the reasons for the solubilizing potential of Labrasol towards polymers.

4. Experimental

4.1. Materials

Eudragit E100, Eudragit L100-55, Eudragit L100, Eudragit S100, Eudragit RSPO and Eudragit PLPO (Degussa India, Mumbai, India), Labrasol (Gattefosse India Ltd., Mumbai, India), Triclosan (Colgate Palmolive India Ltd., Mumbai, India) and repaglinide (Macleod Labs, Mumbai, India) were obtained as donated samples. pEGFP-N2 plasmid (Clontech Laboratories Inc., CA, USA) was purchased for the studies. Double distilled water was freshly prepared before all experiments. All other chemicals were of analytical grade.

4.2. Solubility of various Eudragits in Labrasol

It is not possible to evaluate the equilibrium solubility of polymers in Labrasol. Hence, the ability of Labrasol to solubilize various polymers was evaluated by a method suggested by Devani et al. (2004). Briefly, the selected polymer was added in 5 mg increments to a transparent glass vial containing 1 ml of Labrasol and the vial was shaken at 800 rpm in a water-bath shaker (Remi Instruments, Mumbai, India) at 25 °C for 24 h. The experiment was continued until undissolved polymer was observed visually. All the experiments were performed in triplicate to confirm the results of solubility. The polymers evaluated were Eudragit RSPO, Eudragit RLPO, Eudragit EPO, Eudragit L100-55, Eudragit L100, Eudragit S100 and Eudragit E100.

4.3. Fabrication of Eudragit nanoparticles using Labrasol

Eudragit nanoparticles were fabricated using Labrasol by a nanoprecipitation method. Briefly, the selected Eudragit (50 mg) was dissolved in Labrasol (0.5 ml) using a vortex mixer (Remi Ltd., Mumbai, India). This solution was added drop wise to double distilled water (9.5 ml) under vortexing at 1200 rpm. This results in spontaneous formation of Eudragit nanoparticles.

4.4. Particle size analysis

The average particle size and polydispersity index of the polymeric nanoparticles obtained in various experiments were determined in triplicate by the photon correlation spectroscopy (PCS; Beckman Coulter N4 plus, Wipro, India). Measurements were carried at an angle of 90° at 25 °C. Dispersions were diluted with double distilled water to ensure that the light scattering intensity was within the instrument's sensitivity range. Double distilled water was filtered through 0.45 μm membrane filters (Pall Life Sciences, Mumbai) prior to particle size determination.

4.5. Zeta potential of Eudragit nanoparticles

For evaluation of zeta potential, RLPO nanoparticles were suitably diluted with double distilled water. The zeta potential value of nanoparticles was evaluated in triplicate using a DELSA-Nano (Beckman Coulter, USA).

4.6. Transmission electron microscopy (TEM) of EPO nanoparticles

TEM studies were carried out to evaluate the morphology of the prepared blank EPO nanoparticles. Briefly, nanoparticles were diluted twice with distilled water. The nanoparticles were processed by negative staining**.** The contrast in the micrographs is provided by the heavy metal ion of the negative stain, which strongly scatters electrons. The stain used was uranyl acetate. Briefly, a drop of appropriately diluted nanoparticle dispersion was placed in contact with a Formvar coated 300 mesh size copper grid for 5 min to allow some of the nanoparticles to stick to the grid. The drop of nanoparticle dispersion was drawn off by touching the edge of the grid with a piece of tissue paper held at 90° to the plane of grid. The film was allowed to dry on the copper grid; a drop of 12.5% uranyl acetate stain was then placed on it for 5 min and then drawn off as described earlier. The stained grid was then air dried and examined immediately under the electron microscope (JEM-1010 electron microscope, JEOL, Japan) at various magnifications.

4.7. Fabrication of drug loaded Eudragit nanoparticles

Briefly, an appropriate amount of Eudragit polymer and hydrophobic drug (repaglinide or triclosan) were dissolved in Labrasol (0.5 ml). The drug loaded Eudragit nanoparticles were fabricated as in the process described in section 2.3.

4.8. Entrapment efficiency (EE) of repaglinide and triclosan in nanoparticles

The entrapment efficiency (EE), which corresponds to the percentage of drug encapsulated within and adsorbed on the nanoparticles, was determined by measuring the concentration of free drug (either repaglinide or triclosan) in the dispersion medium. Nanoparticle dispersion (1 ml) was centrifuged at 14000 rpm (Eppendorf Mini-centrifuge) for 20 min to separate nanoparticles. The supernatant was analyzed for unencapsulated repaglinide or triclosan at 242 nm and 282 nm respectively using a validated UV-spectrophotometric method after suitable dilution.

The entrapment efficiency was calculated by the following equation:

$$\%EE = \left[\frac{M_{\text{initial drug}} - M_{\text{free drug}}}{M_{\text{initial drug}}}\right] \times 100 \quad (1)$$

where $M_{\text{initial drug}}$ is the mass of initial drug used for the assay and $M_{\text{free drug}}$ is the mass of free drug detected in the supernatant after centrifuging the aqueous dispersion.

4.9. Ability of Eudragit RSPO and RLPO nanoparticles to condense plasmid DNA (pDNA)

The ability of various RSPO and RLPO nanoparticles to condense pDNA was evaluated using agarose gel electrophoresis. The nanoparticles were filtered through a 0.2 μm nylon filter (Milipore India Ltd., Mumbai, India). The nanoparticles were diluted with sterile water to yield a concentration of 1 mg/ml. The stock solution of pDNA was also diluted to yield a pDNA concentration of 1 mg/ml. The nanoparticle-pDNA complexes were prepared by mixing nanocarriers and pDNA in different concentrations to yield nanoparticles at pDNA ratios of 1:1, 1:5, 1:10 and 1:15 respectively. The final volume was made up to 20 μl with sterile water. The nanocarriers and DNA were incubated for 30 min at room temperature (∼25 °C). DNA condensation was studied using agarose gel electrophoresis. Briefly, samples were loaded into 0.8% agarose gel and were subjected to electrophoresis using a TBE buffer system for 2 h at 5 V/cm. DNA was visualized using ethidium bromide staining. Images were obtained using a UV transilluminator and a Geldoc 2000 gel documentation system (Biorad, Mumbai, India) with the settings adjusted to avoid signal saturation. All the experiments were carried out in triplicate.

Acknowledgements: Authors are grateful to Degussa India, Mumbai, India for donating samples of Eudragit and also to Gattefosse India Ltd., Mumbai, India for Labrasol. The kind help of USV Ltd., Mumbai, India and Colgate Palmolive Ltd., Mumbai, India in donating sample of repaglinide and triclosan respectively is acknowledged. A. Date is grateful to the Indian Council of Medical Research, New Delhi for providing a Senior Research Fellowship.

Authors are also thankful to Deepika Srivastava and Dr. Rita Mulherkar, ACTREC, Navi Mumbai, India for helping with pDNA condensation studies.

References

Asghar LF, Chure CB, Chandran S (2009) Colon specific delivery of indomethacin: effect of incorporating pH sensitive polymers in xanthan gum matrix bases. AAPS PharmSciTech 10: 418–429.

Bucolo C, Maltese A, Puglisi G, Pignatello R (2002) Enhanced ocular anti-inflammatory activity of ibuprofen carried by an Eudragit RS100 nanoparticle suspension. Ophthalmic Res 34: 319–323.

Devani M, Ashford M, Craig DQ (2004) The emulsification and solubilisation properties of polyglycolysed oils in self-emulsifying formulations. J Pharm Pharmacol 56: 307–316.

Djekic L, Primorac M (2008) The influence of cosurfactants and oils on the formation of pharmaceutical microemulsions based on PEG-8 caprylic/capric glycerides. Int J Pharm 352: 231–239.

Dong WY, Maincent P, Bodmeier R (2007) *In vitro* and *in vivo* evaluation of carbamazepine-loaded enteric microparticles. Int J Pharm 331: 84–92.

Elshafeey AH, Kamel AO, and Awad GAS (2010) Ammonium methacrylate units polymer content and their effect on acyclovir colloidal nanoparticles properties and bioavailability in human volunteers Col Surf B 75: 398–404.

Kanikkannan N, Andega S, Burton S, Babu RJ, Singh M (2004) Formulation and in vitro evaluation of transdermal patches of melatonin. Drug Dev Ind Pharm 30: 205–212.

Lieb S, Szeimies RM, Lee G (2002) Self-adhesive thin films for topical delivery of 5-aminolevulinic acid. Eur J Pharm Biopharm 53: 99–106.

Lopedota A, Trapani A, Cutrignelli A, Chiarantini L, Pantucci E, Curci R, Manuali E, Trapani G (2009) The use of Eudragit RS 100/cyclodextrin nanoparticles for the transmucosal administration of glutathione. Eur J Pharm Biopharm 72: 509–520.

Pinto Reis C, Neufeld RJ, Ribeiro AJ, Veiga F (2006) Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine 2: 8–21.

Sha X, Yan G, Wu Y, Li J, Fang X (2005) Effect of self-microemulsifying drug delivery systems containing Labrasol on tight junctions in Caco-2 cells. Eur J Pharm Sci 24: 477–486.

Varshosaz J, Faghihian H, Rastgoo K (2006) Preparation and characterization of metoprolol controlled-release solid dispersions. Drug Deliv 13: 295–302.

Yang ZQ, Xu J, Pan P, Zhang XN (2009) Preparation of an alternative freeze-dried pH-sensitive cyclosporine A loaded nanoparticles formulation and its pharmacokinetic profile in rats. Pharmazie 64: 26–31.

Yen FL, Wu TH, Lin LT, Cham TM, Lin CC (2009) Naringenin-loaded nanoparticles improve the physicochemical properties and the hepatoprotective effects of naringenin in orally-administered rats with CCl(4)-induced acute liver failure. Pharm Res 26: 893–902.

Zhao X, Liu JP, Zhang X, Li Y (2006) Enhancement of transdermal delivery of theophylline using microemulsion vehicle. Int J Pharm 327: 58–64.