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Address of Editorial Board

Faculty of Pharmacy
2, Dunav str., Sofia 1000
Fax (02) 987 987 4

Editor in Chief: ✆(+359 2) 9236 505
E-mail: pharmacia_editor@pharmfac.net
Secretary: ✆ (02) 9236 515
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CONTENTS

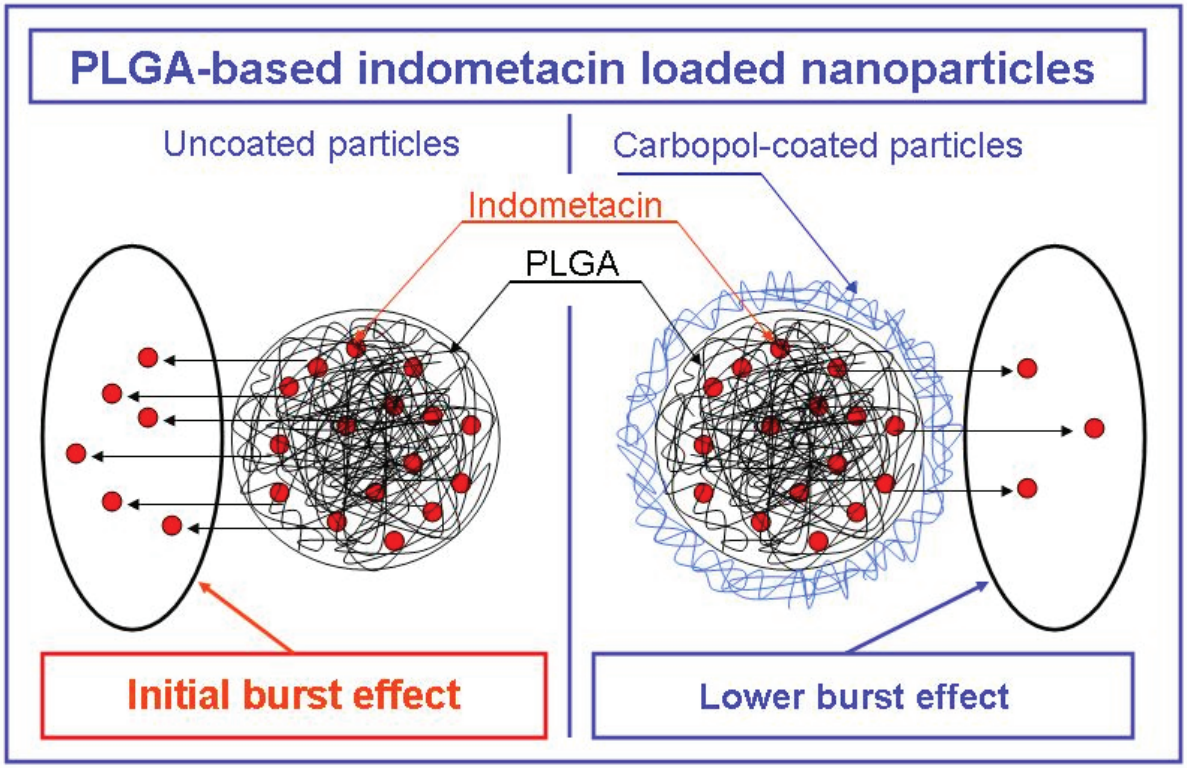
DEVELOPMENT OF CARBOPOL-COATED INDOMETACIN LOADED POLY (LACTIDE-CO-GLYCOLIDE) NANOPARTICLES

B. Tzankov, K. Yoncheva, N. Lambov

Medical University – Sofia, Faculty of Pharmacy, Department of Pharmaceutical Technology and Biopharmaceutics, 2 Dunav str., 1000 Sofia, Bulgaria

Summary: Indometacin incorporation in nanoparticles can reduce the side effects of the drug on the gastrointestinal mucosa. In the present study, indometacin was loaded into poly(lactide-co-glicolide) nanoparticles by simple solvent evaporation method. Nanoparticles possessed average diameter of 500 nm and low polydispersity index (0.2). Variation of the initial ratio between the drug and polymer influenced drug loading degree. The results showed that the increase of the drug concentration resulted in higher loading. The in vitro release profiles in acid and phosphate buffers showed biphasic manner – initial rapid indometacin release followed by slower release for a longer period. The initial burst effect was decreased by coating of nanoparticles with carbopol. In conclusion, the coating of indometacin loaded nanoparticles could be considered appropriate approach for the development of nanosized delivery system with reduced burst release and eventually reduced side effects.

Graphical abstract



Key words: PLGA, nanoparticles, indometacin, carbopol

Introduction

Biodegradable polymeric nanoparticles are promising drugs carriers due to their advantages. These nanoparticles allow sustained release of drugs, reduction in their side effects, increased bioavailability and stability of the drugs [1-3]. Another advantage is that drugs with different solubility could be encapsulated in the biodegradable carriers. Polylactic-co- polyglycolic acid (PLGA) is one of the widely used biodegradable polymers, because of its biocompatibility, biodegradability and non-toxic properties. The ratio of polylactic and polyglycolic acid in the copolymer determines the different rates of drug release. In the body it transforms into its main monomers – lactic and glycolic acid that are easily metabolized and eliminated from the body [4]. One of the main drawbacks of these nanoparticles is associated with the presence of initial "burst" release of the drug substance [5-7]. Among various approaches, coating of nanoparticles with appropriate polymer provides a good opportunity to avoid this problem. Yoncheva et al. have reported that coating of PLGA nanoparticles with chitosan led only to 35% pilocarpine release during the first three hours, whereas for the same time 63% of the drug was released from the non-coated nanoparticles [8]. The properties of the coating polymer could provide different rate and site of the drug release epending on variety of external factors (pH, temperature, etc.).

Indometacin is a nonsteroidal antiinflammatory drug (NSAIDs) with pronounced analgesic effect. It is used for the treatment of inflammation caused by rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and soft tissue injuries such as tendinitis and bursitis. Like other NSAID, the common adverse drug reactions of indometacin are mainly dyspepsia, nausea, vomiting or bleeding from the gastrointestinal tract. Indometacin loading in the sustained-release systems can greatly reduce or even prevent these side effects.

The aim of the present study was to encapsulate indometacin into PLGA nanoparticles in order to achieve sustained release. Further, the coating of the nanoparticles with carbopol could reduce both the initial burst release and the side effect on gastrointestinal mucosa.

Materials and Methods

Materials

Indometacin (2-(1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl) acetic acid and poly(lactide-co-glicolide) (50:50) were provided by Sigma Aldrich. Polyvinyl alcohol (PVA 22000) and carbopol 940 were purchased by Fluka (Switzerland).

Methods

Preparation of particles

The nanoparticles were prepared by solvent evaporation method. Briefly, poly(lactide-co-glycolide) and the active ingredient were dissolved in dichloromethane (5 ml). To this solution aqueous phase containing 1% of PVA (50 ml) was added under stirring (28 000 rpm). Then, the solvent was evaporated at room temperature for 24 hours under constant stirring (600 rpm). After evaporation of the solvent the nanoparticles were separated from the dispersion medium by centrifugation at 15,000 rpm (Hermle Z323K, Germany), washed three times with distilled water and the particles were dried in a vacuum desiccator until a constant mass.

Coating of nanoparticles with carbopol

The coating of the dried nanoparticles was performed by incubation in aqueous carbopol solution (0.012% wt/v). Briefly, 50 mg of nanoparticles were added to 20 ml of carbopol solution and the dispersion was stirred at 600 rpm for 30 min. After incubation, the dispersion was centrifuged and rinsed three times with distilled water. The resulting particles were dried for 72 hours in a vacuum desiccator.

Particle size and zeta potential measurements

The size and zeta-potential were determined by photon correlation spectroscopy and electrophoretic light scattering (Malvern Zetasizer Nano ZS,UK). The nanoparticles were dispertsed in distilled water immediately before measurement (0.5 mg/ml) and measured at scattering angle of 90° at 25°C. The measurements were made in triplicate.

Indometacin loading

Accurately weighed quantity of nanoporticles (10 mg) was poured in 30 ml of methanol. The system is stirred at electromagnetic stirrer at 500 rpm for 60 min and then centrifuged at 10,000 rpm for 10 min. The absorbance of the loaded indometacin was determined spectrophotometrically at 320 nm (Hewllet Packard 8452A, USA).

In vitro release tests

The release of the drug is determined in a shaking air bath (Heidorph Titramax 1000, Germany) at 150

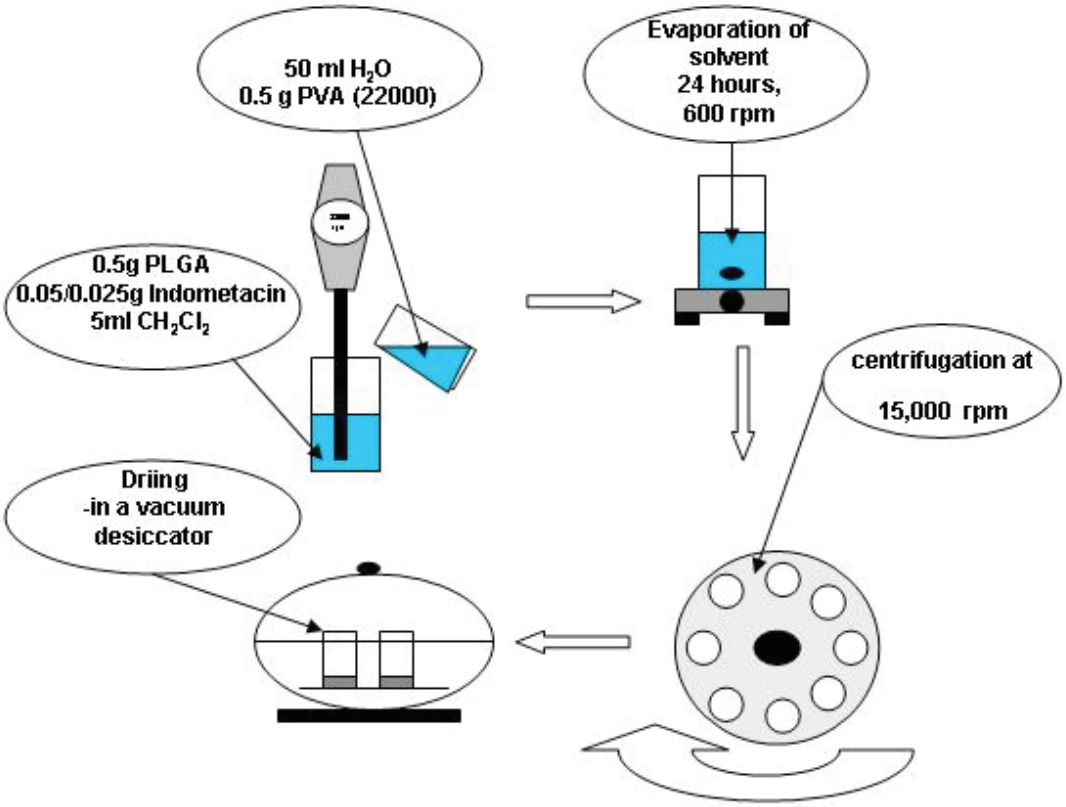


Fig.1 *Preparation of indometacin loaded nanoparticles using solvent evaporation method.*

rpm, 37°C. Studies were carried out in acid buffer (pH = 1.2) and in phosphate buffer (pH = 6.8). Samples were taken at predetermined time intervals and the amount of released drug was determined spectrophotometrically at 320nm (Hewllet Packard 8452A, USA). The concentration of the released substance is calculated by a standard equation (R = 0.997).

Results and Discussion

Preparation of nanoparticles

The most common methods for preparation of PLGA nanoparticles are solvent evaporation, solvent extraction and spray drying [9]. The selection of the most appropriate method depends on the properties of drug and polymer and directly affects the characteristics of the resulting particles.

In our study, the preparation method was selected in conjunction with the properties of indometacin, which is characterized by low water solubility. Simple solvent evaporation method was pointed by numerous studies as the most suitable for the inclusion of drugs with hydrophobic properties [10-12]. Technological scheme of our method is presented in Figure 1. Organic phase containing both the polymer and the drug were emulsified in aqueous phase, followed by evaporation of the organic solvent.

Determination of the main physico-chemical characteristics of nanoparticles

Size and polydispersity

One of the most important parameters influencing the size and polydispersity of the nanoparticles is the speed of agitation during the emulsification. It is well known that with increase of the stirring speed particle size decreases. The speed of agitation in the present study (28000 rpm) resulted in relatively small particle size. As shown in Fig. 2, the size of the nanoparticles was around 500nm. The nanoparticle coating with carbopol slightly increased their size and polydispersity.

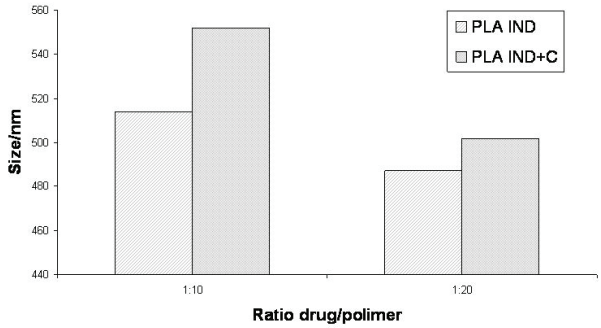


Fig.2 *Particle size of the indometacin-loaded PLGA nanoparticles (nm)*

Zeta potential

Zeta potential of the developed nanoparticles was negative, in particular between -25 and -33 mv (Fig.3). The coating with carbopol did not change the values for the zeta potential compared to non-coated particles.

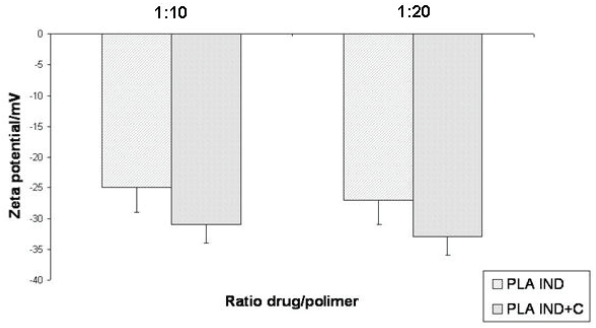


Fig.3 *Zeta potential of the indometacin-loaded PLGA nanoparticles (mV)*

Drug loading

The initial ratio between the polymer and the drug can affect the particle size, the degree of loading and release profile of loaded drugs. For this reason, in our studies two different drug concentrations were investigated -0,05 mg/ml and 0,1 mg/ml, respectively. The results regarding drug loading received at the different concentrations are shown in Fig. 4. As seen, there was statistically significant difference in the loading degree of indometacin depending on the initial concentration. At the lower concentration (0,05 mg/ml) drug loading was about 70%, whereas at the higher concentration (0,1 mg/ml) the drug loading exceeded 85%. These results showed that the increase of the initial concentration of indometacin led to higher loading degree.

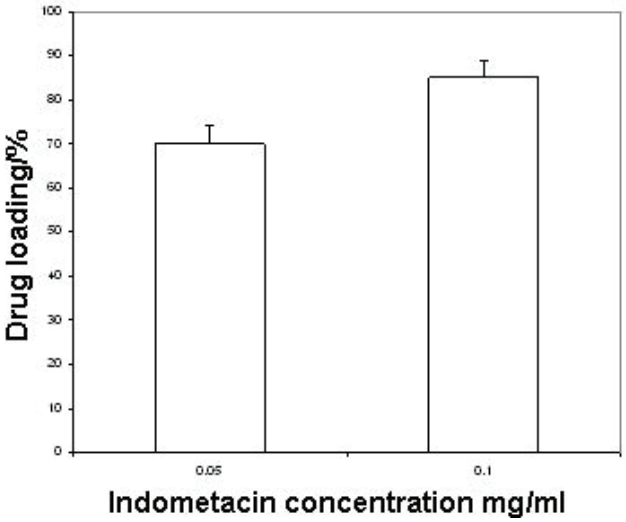


Fig.4 *Influence of the initial concentration of indometacin on the loading degree.*

In vitro release tests

The in vitro release from nanoparticles was studied in buffers with pH 1.2 and 6.8. As shown in Figures 5 and 6, bimodal release profiles were observed. Results showed initial rapid release of indomethacin in both media (55-60% in 1h) and subsequent slow release. This effect was slightly higher for the particles with a lower loading degree. This fact could be due to the fact that a high amount of the drug was located on the surface and near the surface of the nanoparticles.

The nanoparticles were coated with carbopol in order to reduce the initial burst release. The carbopol coating influenced release process in both media. The concentration of indometacin released in 1h from coated nanoparticles was around 15% lower compared to that from non-coated nanoparticles.

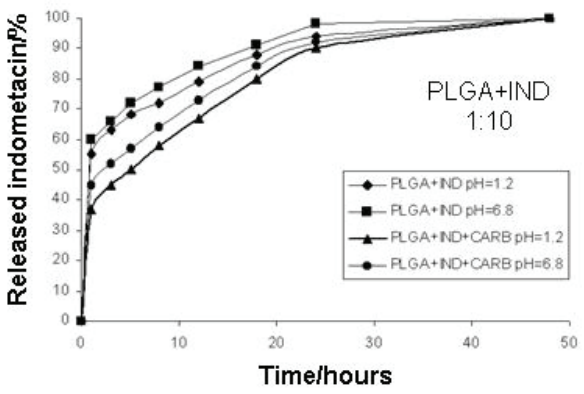


Fig.5 *In vitro release of indometacin from PLGA nanospheres prepared in 1:10 ratio*

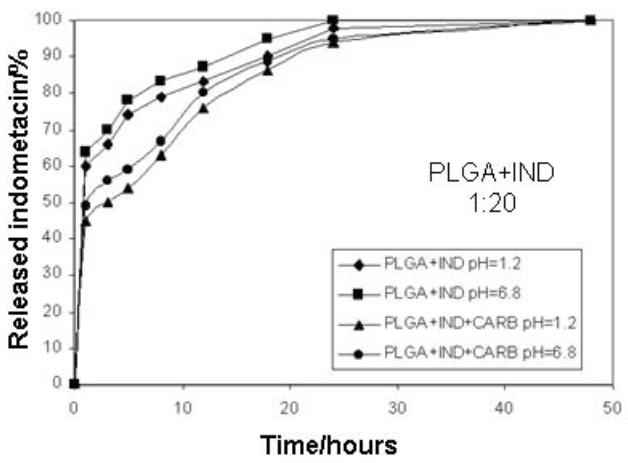


Fig.6 *In vitro release of indometacin from PLGA nanospheres prepared in 1:20 ratio*

Conclusion

Indometacin loaded nanoparticles with an average particle size of about 500 nm were prepared by solvent evaporation method. The method allowed an efficient loading of more than 70% indometacin. Release profiles showed a bimodal pattern - initial rapid release of 60% of the active ingredient and subsequent slow release. The initial burst release of indometacin was overcome by coating of the developed nanoparticles with carbopol. In particular, carbopol coated particles showed significant reduction of the initial burst effect (approximately 15% lower). The decreased initial release would reduce the side effects of indometacin on gastric mucosa after oral administration of nanoparticles.

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✉ **Corresponding author**
Borislav Tzankov
Medical University – Sofia,
Faculty of Pharmacy, Department of Pharmaceutical Technology and Biopharmaceutics,
2 Dunav str., 1000 Sofia, Bulgaria,
e-mail: borislav_tzankov@abv.bg,
Phone: +359888545048