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Research paper

Development and optimization of methotrexate-loaded lipid-polymer hybrid nanoparticles for controlled drug delivery applications

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ABSTRACT

Lipid-polymer hybrid nanoparticles (LPHNPs) are emerging platforms for drug delivery applications. In the present study, methotrexate loaded LPHNPs consisted of PLGA and Lipoid S100 were fabricated by employing a single-step modified nanoprecipitation method combined with self-assembly. A three factor, three level Box Behnken design using Design-Expert® software was employed to access the influence of three independent variables on the particle size, drug entrapment and percent drug release. The optimized formulation was selected through numeric optimization approach. The results were supported with the ANOVA analysis, regression equations and response surface plots. Transmission electron microscope images indicated the nanosized and spherical shape of the LPHNPs with fair size distribution. The nanoparticles ranged from 176 to 308 nm, which increased with increased polymer concentration. The increase in polymer and lipid concentration also increased the drug entrapment efficiency. The *in vitro* drug release was in range 70.34–91.95% and the release mechanism follow the Higuchi model ($R^2 = 0.9888$) and Fickian diffusion ($n < 0.5$). The *in vitro* cytotoxicity assay and confocal microscopy of the optimized formulation demonstrate the good safety and better internalization of the LPHNPs. The cell antiproliferation showed the spatial and controlled action of the nanoformulation as compared to the plain drug solution. The results suggest that LPHNPs can be a promising delivery system envisioned to safe, stable and potentially controlled delivery of methotrexate to the cancer cells to achieve better therapeutic outcomes.

1. Introduction

Chemotherapy is considered as an effective approach for the management of various cancer diseases (Zheng et al., 2015). The challenging task is to deliver chemotherapeutic agents to cancerous cells without influencing the normal tissues (Sharma et al., 2013; Yingchoncharoen et al., 2016). The low aqueous solubility of therapeutics agents, lack of specificity, and dose dependent side effects limit the conventional systems, thus requiring the newer novel approaches to address these limitations (Peer et al., 2007). Rapid removal or limited influx of different chemotherapeutic agents through various efflux pumps contribute to development of multi drug resistance (Wakaskar, 2017b). Similarly, the precise delivery of the active pay load at the required site without crossing the threshold or potentially toxic level. Such problems associated with the 5-fluorouracil has been addressed with the dendrimers conjugated with the polyethylene glycol (Wakaskar, 2017a; Wakaskar et al., 2015). The design of such systems involve many interrelated processes and mechanisms toward the successful fabrication and optimization in term of physicochemical, physiological and pharmacological properties (Woitiski et al., 2009).

Similarly, the complex chemical composition of these nanoparticles required a lot of experiments to check the influence of their structural components on the aforementioned properties of the drug delivery systems. Different experimental and statistical designs have been employed for the development and optimization of the various NPs formulations (Fanaie et al., 2016; Khajeh, 2009). Among these, Response Surface Methodology (RSM) provides a set of different statistical tools that can be employed to improve the products and process parameters

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(Pizzol et al., 2016). These experimental designs have the advantages of less number of trials for optimization, saving time and providing the data on the influence of individual and combined effects of the formulation, and the process variables on the response variables to obtain the optimized formulation (Gajra et al., 2015b; Kashif et al., 2016).

In the last decades, nanotechnology has emerged as the most prominent tool in the development of novel drug carrier systems, providing versatile clinical applications and scale-up for industrial production (Allen and Cullis, 2013). Polymeric nanoparticles and lipid nano-carriers (*e.g.*, solid lipid nanoparticles and/or liposomes) are two distinct drug delivery approaches, which have been approved by US FDA for clinical use (Sanna et al., 2014). Different polymers, such as poly(D, L-lactide-co-glicolide), polycarprolactone and chitosan have been used to prepare nanoparticles that demonstrate the effective delivery of several therapeutic agents (Kumar et al., 2017; Öztürk et al., 2017). These polymeric nanoparticles provided better drug loading, biodegradability and stability characteristics, whereas the lipid nanocarriers offer the use of natural and synthetic lipids with better biocompatibility, long circulation half-life and easy surface functionalization (Huo et al., 2015; Zheng et al., 2015). Doxil® (Doxorubicin liposome), and Abraxane® (Albumin coated Paclitaxel) are examples of such delivery systems (Chelopo et al., 2017; Zhang et al., 2017b). Kim et al. prepared the alkanethiol coupled gold nanoparticles for the encapsulation of highly hydrophobic drug tamoxifen with high payload and better stability in the plasma (Kim et al., 2009). However, each of these systems has some drawbacks in terms of rapid drug diffusion and leakage, non-specific release, dose related toxicities and uncontrolled drug release (Dehaini et al., 2016).

Recently, polymeric and lipid based nanocarriers have been merged together to integrate the positive attributes and to overcome the possible drawbacks in the form of lipid-polymer hybrid nanoparticles (LPHNPs) (Sengel-Turk and Hascicek, 2017). Lipid-polymer hybrid (LPH) system has overcome the problems of rapid drug diffusion, non-specific release, dose related toxicities and uncontrolled drug release (Zhang et al., 2008). This system comprises three distinct components: (i) inner most polymeric core enclosing the active therapeutic moiety; (Tahara et al., 2017) lipid layer surrounding the polymeric core material; and (iii) outer most PEGylated lipid covering that increase the particle retention time inside the body (Yan et al., 2015). This unique structural design provides the mechanical integrity by maintaining the particle size, good biocompatibility and *in vivo* stability, optimized drug entrapment, loading of multiple drugs of different physicochemical properties and functionalized, targeted and controlled delivery of these therapeutic moieties at the active site (Mandal et al., 2016). The surface of these particles have been modified using the carbodiimide click chemistry or thiol-maleimide approach with the folic acid, aptamer and different peptides (Wakaskar, 2017c). Among those drugs, methotrexate (2, 4-diamino-N10-methyl propylglutamic acid, MTX), a folic acid analogue, has been used for the treatment of various cancers such as breast cancer, brain cancer, ovarian cancer and different leukemia (Abolmaali et al., 2013). However, the low solubility profile, dose dependent toxicity, shorter half-life and cellular efflux restrict the efficient therapeutic use of MTX (Seo et al., 2009).

Taking altogether, the present study was designed to develop and optimize the MTX-loaded LPHNPs by using the combination of lipid and polymer through the statistical design expert approach. Poly(lactic-co-glycolic acid) (PLGA) was used as the polymer for the encapsulation of the drug because of its biodegradable nature and potential for high loading of hydrophobic drugs (Chan et al., 2009) and phospholipid (Lipoid S100) as a natural lipid for coating the polymeric core which mimic the biological membranes and help in better penetration of the nanoparticles. A single-step modified nanoprecipitation method combined with self-assembly was employed to fabricate LPHNPs (Zhang et al., 2015a). Furthermore, Design-Expert® was used to get a set of formulations keeping the formulations variable at three levels (Pardeshi et al., 2013). The response variable, such as particle size, drug entrapment and release in a controllable manner were defined to obtain an optimized formulation. The impact of the formulation variable (individual and combined effect) was also determined in order to get an optimized formulation. In addition, the physicochemical characterization of the LPHNPs, the MTX-loaded LPHNPs release profiles, and *in vitro* studies in different cancer cells were also assessed for the developed LPHNPs.

2. Materials and methods

2.1. Materials

MTX was purchase from Tokyo chemical industry Co. Ltd, Japan. Poly (D, L-lactide-co-glicolide) (PLGA, PURASORB®) with a 50:50 monomer ratio was obtained as a kind gift from Purac biomaterials, Netherlands. Lipoid S-100 (Phosphatidylcholine from soybean) was obtained as a kind gift from the Lipoid GmbH, Ludwigshafen, Germany. Lutrol® F-68 was purchased from BASF Crop. Ludwigshafen, Germany. Dimethylformamide (DMF) and Sodium phosphate dibasic (Na_2HPO_4) were purchased from Sigma Aldrich, St. Louis, MO, USA. Citric acid was purchased from Hawkins INC. Minneapolis, MN, USA. Hank's balance salt solution (HBSS), trypsin (2.5%), Dulbecco's Modified Eagle's Medium (DMEM), Dulbecco's phosphate buffer saline (10 × PBS), Roswell Park Memorial Institute (RPMI), L-glutamine (200 mM), fetal bovine serum (FBS), nonessential amino acids (100 × NEAA) and penicillin-streptomycin (100 × PEST) were all purchased from HyClone (U.S.). The MDA-MB231breast carcinoma cells and PC3 prostate cancer cells, and HT29 colorectal carcinoma cells were obtained from American Type Culture Collection. Milli-Q water (Merck Millipore, USA) was used in the preparation of formulation and buffers. All the other chemicals, ingredients and the reagents were of analytical grade and the solvents used were of HPLC grade.

2.2. Methods

2.2.1. Experiment design for optimization

The Design-Expert® 7.0.0 software employing 3 factorial, 3 level Box-Behnken statistical design suggested 15 runs to optimize the LPHNPs. Polymer concentration (X1), lipid concentration (X2) and surfactant concentration (X3) were considered as independent variables. Whereas, particle size (Y1), entrapment efficiency (Y2) and percent drug release (Y3) were taken as dependent (response) variables. Each independent variable varies in triplicate manner, including higher, middle and lower values as given in Table 1. These values were determined on the basis of initial trial experiments and the review of available literature. The design matrix consisting of 12 factorial and 3 center points with a set of 15 formulations. The optimization of independent variables (X1, X2, X3) was aimed to maximize the entrapment of the system (Y2), prolong the release time (Y3) and to minimize

Table 1
Levels of independent and dependent variables in the experimental design.

Independent variables		Levels		
		Maximum (+1)	Middle (0)	Minimum (−1)
X1	Polymer Concentration (mg/ml)	4	3	2
X2	Lipid Concentration (mg/ml)	3	2	1
X3	Surfactant Concentration (%)	1.0	0.75	0.50

Dependent variables		Desired Outcomes
Y1	Particle Size	Minimized
Y2	Entrapment Efficiency (%)	Maximized
Y3	Drug Release (%)	In range