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High-throughput *in vitro* drug release and pharmacokinetic simulation as a tool for drug delivery system development: Application to intravitreal ocular administration

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ABSTRACT

In vitro estimation of release kinetics from drug delivery systems is needed in formulation development. Cost-effective methods of assessment for delivery systems are needed particularly in the case of biologicals and drug administration routes that are difficult to screen *in vivo* (*e.g.* intraocular drug delivery). As a proof-of-concept, we demonstrate here a practical high-throughput methodology to investigate *in vitro* drug release and predict resulting drug concentrations in the eye after intravitreal administration. 96-well plate based assay aided with robotic sampling was used to study release of eight model drugs of varying physicochemical properties (dexamethasone, vancomycin, alpha-lactalbumin, lysozyme, myoglobin, albumin, lactoferrin, human IgG) from twelve alginate microsphere formulations. The amount of drug released over a period of time was assessed by photometric and fluorescence methods. *In vitro* drug release rates obtained were used in pharmacokinetic simulations using one-compartment model of the vitreal cavity with anatomical volume of distribution and clearance estimates based on the literature precedence. An integrated approach of drug release screening and pharmacokinetic simulations can prove to be a useful methodology in guiding formulation development for ocular delivery in animal models. In general, the methodology has the potential to be a cost-effective tool for early stage drug delivery system discovery and development.

1. Introduction

Drug delivery systems have emerged at the frontiers of biomedical research and innovation. The advent of smart drug delivery options has changed the landscape of drug development, particularly in the case of peptides and proteins, that are rather difficult to deliver (Sinha and Trehan, 2003; Brown, 2005; Pisal et al., 2010; Gupta et al., 2013). Controlled drug delivery is important in the administration of drugs with narrow therapeutic index, and for the prolongation of drug effect (Uhrich et al., 1999; Andreopoulos, 2003). Potential benefits of drug delivery systems include target specific delivery, prolonged retention and activity, controlled release to maintain drug concentration at desired levels, reduction of drug toxicity and protection of drug against degradation in the body fluids. Various types of delivery systems exist and are generally classified based on their chemical composition, shape and size. Liposomes, microcapsules, nanoparticles, lipid matrices, microparticles and hydrogels are some of the commonly used drug delivery systems (Tiwari et al., 2012; Zhang et al., 2013).

Drug release profile from delivery systems is dependent on many different factors; it is multivariate in nature. The release profile depends on interactions of the drug with the polymer matrix; stability, physicochemical properties of the drug encapsulated delivery system and environmental conditions of release. An ideal delivery system should allow controlled release of the drug at the desired site to have maximum efficacy and minimal toxicity. *In vitro* drug release studies are carried out to test release profile of drugs from formulations as a part of preclinical development of delivery systems. Based on the observed release profile, the formulations are modified to achieve desired release profiles. This process is iterative and optimization can be an extensive exercise.

Typically, in a drug release experiment, the delivery system is suspended in a buffer solution, incubated at desired temperature

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and the amount of drug release is determined periodically with appropriate analytics. The methodology is suited to test formulations 'one-at-a-time' which can be time consuming and costly. Since in the early developmental phase, most experimental drugs (small molecules and biologics) are limited in supply and expensive, a high-throughput miniaturized release assay is desirable. Even though drug release from a delivery system depends on interplaying factors, systematic investigations of the effects of medium and formulation components on drug release have been limited. In recent past, combinatorial synthesis of thousands of lipid-like polymers for siRNA delivery (Mora-Huertas et al., 2010; Love et al., 2010) and a small library of nanoparticles with unique small molecules on the surface (Weissleder et al., 2005) has been achieved. Production of combinatorial polymer and nanoparticle libraries necessitates new approaches to analyze release kinetics in a cost effective and timely manner. It is also important to carry out release studies in the presence of biological constituents that are present at the site of administration. The biological constituents and media are precious materials that have limited availability. In a recent report, solubility of various drugs was analyzed in real human intestinal and artificial release media (Heikkilä et al., 2011). A miniaturized high-throughput *in vitro* release assay would not only require smaller amounts of the therapeutic drugs and delivery systems but would also allow flexible modification and testing of different release conditions (including spiking with biological fluids and components).

Pharmacokinetic (PK) simulation with the *in vitro* drug release data potentially extrapolates to the *in vivo* context by estimating drug elimination from the compartment of interest. Drug concentration over time for a given release rate from the formulation can be assessed when experimental release profiles are sequentially linked to drug distribution and elimination characteristics at the site of interest. The predicted concentrations can be compared to the desired concentration required for therapeutic efficacy; such estimates are valuable for reformulating the drug delivery system.

We report here a 'proof-of-principle' methodology of high-throughput *in vitro* drug release studies and PK simulations for ocular drug delivery. Multi-well based drug release screening was carried out using eight drug-like molecules in twelve alginate based microsphere formulations (in total 96 combinations). Alginate microspheres were chosen because they are easy to prepare, biocompatible and can be modified with divalent ions as cross linkers. The expected free drug concentrations in the vitreal cavity of the eye were simulated for intravitreal microsphere administration.

2. Materials and methods

2.1. Fluorescent labeling of proteins and vancomycin

Proteins were labeled with fluorescein isothiocynate (FITC). Briefly, a 20-fold molar excess of FITC in anhydrous DMF was added to protein solutions (2 mg/ml) in 100 mM carbonate buffer. The reaction mixture was gently agitated for 90 min at room temperature protected from light. The dye was removed and the labeled proteins concentrated by centrifuging the reaction mixture using spin filters having 5 kDa molecular weight cut off. Labeled vancomycin (BODIPY FL–Vancomycin) was purchased from Invitrogen.

2.2. Preparation of alginate microspheres

Alginate microspheres were made using an in-house designed apparatus detailed elsewhere (Kontturi et al., 2011). Briefly, microspheres were prepared by dispersing alginate solution (UP, LVG, FP-303–02; Novamatrix, Norway) extruded with nitrogen gas through a nozzle that supports co-laminar flow. Alginate solution was pumped through a syringe where the motor driven plunger movement was precisely controlled by a computer-interfaced system. HPLC needles used for alginate extrusion were purchased from Hamilton (gauge 31; outer/inner diameter: 0.26 mm/0.13 mm; point style 3 with blunt end). Blank microspheres were prepared by dispensing alginate solutions (1.5% and 1.0%) into cross-linking solutions: calcium chloride ($CaCl_2{\cdot}2H_2O$, Riedel-de-Haen, Germany); barium chloride ($BaCl_2{\cdot}2H_2O$, Sigma–Aldrich, USA); $CaCl_2/BaCl_2$ (60:40); $BaCl_2/CaCl_2$ (60:40). The formed microspheres were incubated in the respective cross-linking solutions for 30 min with gentle agitation.

The spherical blank microspheres were on an average 200 μm, as determined by an optical microscope. The microspheres were transferred to 96-well plates in replicate. Equal amounts of microspheres of the same formulation (556 μg/well for 1.0% and 833 μg/well for 1.5%) were transferred to the wells of a Multi-Tier™ plate system (Laboratory Supply Distributors, Corp., New Jersey, USA). The plate system has provision for fitting 96 disposable 2.0 ml glass vials. The microspheres were washed with 25 mM Hepes (pH 7.4) buffered saline (0.9%) followed by saline solution (0.9%). Poly-L-lysine (PLL) coated microspheres were prepared by adding 0.1% w/v PLL hydrobromide (Sigma–Aldrich, USA) solution to the blank microspheres and gently agitating for 30 min. The PLL coated microspheres were then treated with 0.05% w/v sodium alginate (30 min, gentle shaking) to neutralize any excess positive surface charge.

The microspheres were then washed with 0.9% saline solution followed by phosphate buffered saline (PBS, Gibco® UK), pH 7.4. PBS washings were removed and 200 μl of model drug solutions were added to the microspheres and left to incubate overnight. Amounts of model drugs used for loading the microspheres were as follows: 300 μg of dexamethasone phosphate; 200 ng of vancomycin; 7.5 μg of α-lactalbumin, lysozyme and myoglobin; 5 μg of bovine serum albumin (BSA), lactoferrin and human IgG (hIgG). Unloaded (excess) amounts of drugs were removed by three serial washings (1.4 ml, 1.6 ml and 1.6 ml) with PBS and estimated by fluorescence and photometric based methods. The percentage amounts of drug encapsulated in alginate formulations have been listed in Table S1 (Supplementary material).

All steps of washings, PLL coating and drug loading were carried out with an automated liquid handling workstation, Biomek NX^P (Beckman Coulter Inc., USA) equipped with an eight channel head.

2.3. Drug release assay

2.0 ml isotonic phosphate buffered saline (PBS, Gibco® UK) having phosphate ion concentration of 8 mM and pH 7.4 was added to each well containing the microspheres. The plates were incubated at 37 °C with gentle agitation (50 rpm). Care was taken to seal the plates tightly and place them in a closed chamber containing PBS to minimize the evaporation. PBS (1.6 ml) was sampled at periodic intervals to determine the amount of drug released and an equal volume added back as replacement. Sampling was carried out with the liquid handling workstation, Biomek NX^P.

Concentration of dexamethasone phosphate was measured by recording the absorbance at 241 nm; concentrations of BODIPYL-vancomycin and FITC labeled proteins were estimated by measuring fluorescence at Ex./Em.: 504 nm/511 nm and 494 nm/517 nm, respectively. Photometric and fluorescence measurements were made using the multiwell plate reader, Varioskan Flash (Thermo Scientific, USA).

2.4. Pharmacokinetic simulations

The expected drug concentration profiles in the ocular vitreous were simulated for microsphere administration as intravitreal