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Research article

PARTITION-OPTIMIZED SINGLE EMULSION PARTICLES IMPROVE SUSTAINED RELEASE OF AMPHIPHILIC BUMPED KINASE INHIBITORS TO CONTROL MALARIA TRANSMISSION

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ABSTRACT: Amphiphilic molecules are challenging to be incorporated into polymeric particles for sustained release due to their significant solubility in both water and organic solvent used in the fabrication process. Here, we investigated an extensive panel of fabrication methods for the incorporation and release of amphiphilic molecules, in particular, novel amphiphilic bumped kinase inhibitors (BKIs). Previously, BKIs were shown to reduce malaria transmission by blocking of gametocyte exflagellation. Prolonged BKI bioavailability for effective transmission blocking is crucial since infectious gametocytes circulate for several weeks in the mammalian host, well beyond the half-life of BKIs. So far, delivery systems for sustained release of those BKIs have not been successfully formulated yet. Here we demonstrate that out of several delivery vehicles the partition-optimized single emulsion particles are the ideal system for incorporation and sustained release of amphiphilic BKIs. They increased the incorporation greater than 90% through optimized partitioning of amphiphilic molecules to the polymer phase and sustained release of BKIs up to several weeks with a reduction in the initial burst release. Overall this work provides a method for the incorporation and sustained release of amphiphilic BKIs, and can be adapted for other amphiphilic molecules.

Key words: Amphiphilic; malaria; Bumped kinase inhibitors; Particles; Sustained release.

INTRODUCTION

Malaria is a mosquito-borne parasitic disease, resulting in over 200 million clinical cases and 1.4 million-recorded deaths per year (91% of these deaths are in the African region and 86% in children) (WHO 2014). Since essentially all cases of malaria are via mosquito to human routes, eliminating this stage of parasite transmission can eradicate the disease. Currently, most *falciparum* malaria treatments focus on lessening the burden of the asexual parasite in the blood stage and do not have a direct effect on transmissible stages, i.e., gametocytes (Baker DA 2010). The two drugs with anti-gametocyte activity, primaquine and artemisinin combination therapy (ACT), are imperfect at reducing malaria transmission to mosquitoes. In the case of primaquine where mature gametocytes of *Plasmodium falciparum* can be cleared quickly, its clinical use has been limited due to problems such as hematological toxicity in patients with glucose-6-phosphate dehydrogenase deficiency while ACT kills immature but not mature gametocytes (Bousema T, Drakeley C (2011). Additionally, although treatment is helpful, current malaria drugs are too expensive for many malaria epidemic areas where only 42% of the public can pay for ACT and only 16% of children actually receive ACT, demonstrating the importance of malaria control and eradication (. Achan J et al, 2011, Greenwood BM 2008). We recently described the pyrazolopyrimidine-based bumped kinase inhibitors (BKIs), which block the male gametocyte exflagellation step by inhibiting *Plasmodia* calcium-dependent protein kinase 4 (CDPK4) in mosquitos (Ojo KK et al, 2012, 2014, Vidadala RS et al, 2014).

In a mouse malaria transmission model (*Plasmodium berhei*), this inhibits the development of parasites inside the mosquito gut after a blood meal was taken from a malaria-infected mouse and in *P. falciparum* infected human blood mixed with BKIs. BKIs can be administered at high concentrations with minimal toxic effects and almost no activity against mammalian kinases (Ojo KK et al, 2010, 2012, 2014). A major challenge to the practical application is the rapid clearance of BKIs from the blood stream when administered via oral dosage or intraperitoneal injection. Inhibition of parasite exflagellation events are reversed in the absence of CDPK4 inhibiting BKIs and transmission of malaria to mosquitoes is resumed (Ojo KK et al, 2012).

Additionally, since viable and infectious gametocyte-stage parasites persist in mammalian hosts for at least 21-28 days after treatment, even in the absence of clinical symptoms, longer exposures of transmission-blocking drugs are needed to effectively block malaria transmission after therapy, but have been challenging for delivery systems due to the BKIs amphiphilic nature. Two BKIs, BKI-1 and 1294 were chosen due to similarities in molecular weight and chemical properties, except that the latter contains an additional methyl group on the secondary amine of the 4-*piperidine*. This modicum alteration adds a slight hydrophobicity to the amphiphilic BKI and has previously been shown to contribute to the reduction of oxidative metabolism of the *piperidine* ring by liver microsomes *in vitro* (Ojo KK et al, 2014). This diminution in metabolism reduces the overall blood clearance rate of 1294, but levels are still insufficient to suppress transmission up to 28 days. A drug delivery platform that can provide sustained release of BKIs into the bloodstream, and maintain effective blood level for >28 days after treatment would be an essential public health tool for malaria transmission control. However, amphiphilic molecules like BKIs are difficult to incorporate into basic particle systems because they do not possess a strong affinity to either the aqueous or organic phase, making these commonly used particle fabrication methods hard to utilize to achieve high incorporation. This paper addressed these challenges by investigating several efficient sustained release particle fabrication methods with the goal of prolonging an effective level of the slightly different amphiphilic inhibitors BKI-1 and 1294 in the bloodstream.

MATERIALS AND METHODS

Materials

Poly (DL-lactide-co-glycolide) (PLGA) resomer (50:50 ester-terminated with IV 0.55–0.75) was purchased from Lactel Absorbable Polymers (Cupertino, CA). BKI-1 and 1294 bumped kinase inhibitors were synthesized as earlier described (Johnson SM et al, 2012). PLGA/BKI solutions were prepared by dissolving 10-50 mg/mL PLGA and 2–100 µg BKI/mg PLGA in solutions as indicated. Dichloromethane (DCM), dimethyl sulfoxide (DMSO), and glycerol was purchased from EMD (EMD Chemicals, Inc., Gibbstown, NJ). Poly vinyl alcohol (PVA) and additional chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Particle fabrication

For System I–solid dispersion/single emulsion particles were created using a single emulsion nanoprecipitation method utilizing a slightly miscible solvent to assist in precipitation [11-13]. Briefly, BKI-1 or 1294 were dissolved directly in 1 mL of PLGA/DCM solution or 1mL PLGA/DCM/DMSO/water solution. The solutions were vortexed with the Vortex Genie 2 (Scientific Industries, Bohemia, NY) at a medium-high speed, then sonicated in a water bath approximately 5 times at 5 minutes each. The solution was then poured into 2 mL of 1% PVA in a beaker and magnetically stirred for 3–4 hours. The drug-containing PLGA/DCM solution was sonicated for 10 seconds twice at output 7 with Model 3000 Ultrasonic Homogenizer (Biologics, Inc., Cary, NC).Two milliliter of 5% PVA was added drop-wise during vortexing and sonication. The solution was poured into 2 mL of 5% PVA during vortexing and sonication. Then, the solution was poured into 2 mL of 1% PVA in a beaker and magnetically stirred for 3-4 hours. For System II – double emulsion particles were created by dissolving BKIs in different ratios of DMSO: water or DMSO: glycerol and sonicated into a solution of PLGA/DCM. Further PVA addition and sonication steps were performed as described above. For System III – core/shell particle, two types of particles were created: 1) zwitterionic polymer-shell was fabricated by co-blocking PLGA with butyl methacrylate-propylacrylic dimethylaminoethyl methacrylate (BMA-PAA-DMAEMA) in DCM prior to addition of BKIs, and 2) alginate-core by mixing BKIs into alginate gelling solution prior to emulsifying in PLGA/DCM mixture. All particles were collected by centrifuging the last solution at 12,000 rpm for 10 minutes using a Sorvall Legend RT centrifuge (DJB Labcare Ltd. Buckinghamshire, England), and three times at 10,000 rpm for 10 minutes using an Eppendorf Centrifuge 5415D (Eppendorf, San Diego, CA) with resuspensions in MilliQ water. Particles were last re-suspended in 200–800 uL MilliQ water depending on particle concentration. Samples were stored at 4^{o}C if not used for testing immediately.

For measuring the total drug incorporated into particles, samples were lyophilized overnight using a Freezone 4.5 Freeze Dry System (Labconco Corporation, Kansas City, MO).

Particle fabrication via fluidic nanoprecipitation

For the fluidic NPS, particles were fabricated based on an adapted system [14]. The initial solution of approximately 1 mL BKI-1/PLGA or 1294/PLGA was injected into a flowing stream of dispersant, which was immediately collected into a beaker, and magnetically stirred for 3–4 hours. A stainless steel needle (BD-305127 25 ½ G) was inserted approximately 3/32" into a 12" length of Vincon tubing (ID 1/8", OD 1/4") where the dispersant phase flew through. The inhibitor/PLGA solution was fed through the needle at 5 mL/min using a 3 mL syringe controlled by a syringe pump (KD Scientific, Holliston, MA). The dispersant channel was 1% PVA adjusted to a given pH flowing at 60 mL/min controlled by a Variable flow peristaltic mini-pump (Fisher Scientific, Hampton, NH). Particles were collected, centrifuged, and stored as described in the preceding section.

Characterization of the particles

Particles were resuspended into 10 mM KNO_3 solution. Particle size, polydispersity, and zeta potential were measured using a Zetasizer Nano-ZS (Malvern, Worcestershire, United Kingdom).

Additionally, scanning electron microscope (SEM) was used to examine the size, size distribution, and morphology of the particles. For SEM, particles were dried overnight on a silicon wafer, and then sputter-coated with gold using a SPI Sputter Coater (Structure Probe, Inc., West Chester, PA). Images were taken with a JEOL7000F SEM with a beam voltage of 5–10 kV (Electron Microscopy Center, University of Washington, Seattle, WA). Particle diameter was measured 2–3 times per particle and approximately 60–500 particles per sample were randomly chosen. Transmission electron microscope (TEM) was used for internal distribution experiments with quantum-dot loaded particles fabricated as described above.

Measurement of total active inhibitor incorporated

Lyophilized particles were completely dissolved in DMSO to produce between 1–5 mM of estimated drug content and centrifuged to remove any remaining polymers. Drug concentration in each sample was measured and calculated by comparing with the inhibition concentration-dependent curve with a known concentration of inhibitors. Samples were assayed for the inhibition of *Pf*CDPK4 via a luminescent readout kinase inhibition assay. Assay reactions were performed with 10 µM ATP, 40 µM Syntide-2 (PLARTLSVAGLPGKK) (American Peptide Company, Inc. Sunnyvale, CA), 146.5 nM*Pf*CDPK4, in 20 mM HEPES (pH 7.5), 0.1% BSA (w/v), 10 mM $MgCl_2$, 1 mM EGTA, with or without 2 mM $CaCl_2$ (Ojo KK et al, 2014, Vidadala RS et al, 2014). The assay evaluates protein kinase activity of *Pf*CDPK4 based on changes of ATP concentration in the presence of peptide substrate and a series of dilutions of samples and known concentrations of inhibitors. Both the total drug incorporated and the time-release samples were tested as the method described above. Data was analyzed using Prism software to determine the concentration of inhibitors that give 50% of reduction in enzyme activity (IC_{50}) graph with known concentration of pure BKI-1 or 1294 as a positive control. Values for total amount of BKI in particles were calculated using the following equation (where I = amount of drug in particles (ug), C_{BKI}= starting concentration (estimated, (mM)), D = dilution factor, usually 1000x unless otherwise indicated, $IC50_{RM1}$ = soluble drug concentration for 50% inhibition (µM), $IC50_{NP}$ = drug in nanoparticle concentration for 50% inhibition (µM), V = volume used to dilute particles (L), MW = molecular weight of drug (µg/µmol)):

$I = C_{BKI}XDX (IC50_{BKI} / IC50_{NP}) XVXMW_{BKI}$

In *Vitro* Release

Particles (approximately 1–10mg) were resuspended in 100 uL of sterile PBS (pH 7.4) and incubated in a 37°C water bath. Buffer was utilized to mimic the conditions of particles administered subcutaneously. Samples were taken at between 0 and 12 hrs on day 0, daily until day 7, and weekly after day 7.At each time point, samples were centrifuged for 10 minutes at 10,000 rpm. The entire supernatant was removed and replaced with 100 uL of fresh PBS. Samples were returned to the 37°C water bath. The amount of drug released, cumulative drug release, and percent release from each sample were calculated based on the *Pf*CDPK4 kinase activity inhibition data obtained by the comparative kinaseglo assay, previously described (Vidadala et al., 2014; Ojo et al. 2012). Minimum reliability of inhibition, or the minimum effective concentration (MEC) was set at 20% or 0.2 ng of BKI-1 or 1294 for most samples Values for release of BKI from particles (R) were calculated using the following equation (whereI_{NP} = percent inhibition of drug from nanoparticle, I_{BKI} = percent inhibition of soluble drug, $IC50_{BKI}$ = soluble drug concentration for 50% inhibition (µM), D = dilution factor, usually 1000x unless otherwise indicated, V = volume used to dilute particles (L), MW = molecular weight of drug (ug/umol)):

$R = I_{NP}X (IC50_{BKI} / I_{BKI}) XDXVXMW_{BKI}$

Partition Optimization

Partition coefficient was analyzed utilizing a standardized shake-flask method from the Office Journal of the European Communities, 2002 (Comm JE 2002). Briefly, BKI-1 or 1294 were dissolved in mixtures of DCM and water (140 uL of DCM and 860 uL of water) at pH = 6, 8, 10, and 12.Solutions were vortexed and allowed to separate for approximately 2 hours.

Samples were taken from the organic layer and the aqueous layer and processed via an IC_{50} inhibition assay for total inhibitor content. Partition coefficient (P), the ratio of molar concentration of inhibitor in organic phase to concentration of inhibitor in aqueous phase, was analyzed from these results. For pH testing, the pH of aqueous solutions used at various stages of the particle fabrication process was altered between pH 6.4 – pH 12.The stages included: 1) the internal phase pH for preliminary double emulsion systems, where the inhibitor was dissolved in an aqueous phase prior to addition to the organic polymer phase, 2) the fabrication step for both non-fluidic and fluidic systems, which is the aqueous continuous phase surrounding particles during fabrication (i.e. the high-velocity channel during fluidic nanoprecipitation and the aqueous continuous phase during non-fluidic nanoprecipitation), and 3) the recovery step for both non-fluidic and fluidic systems, which is the aqueous solution used to wash the particles after fabrication.

RESULTS AND DISCUSSION

Investigation of an Extensive Panel of Particle Fabrication Methods for Amphiphilic Incorporation

Multiple types of sustained release particles were tested for the amphiphilic bumped kinase inhibitor (BKI) incorporation. The particles were classified into three systems: System I including solid dispersion and single emulsion particles, System II including double emulsion particles, and System III including core/shell particles. Schematic of the different systems are in Figure1.

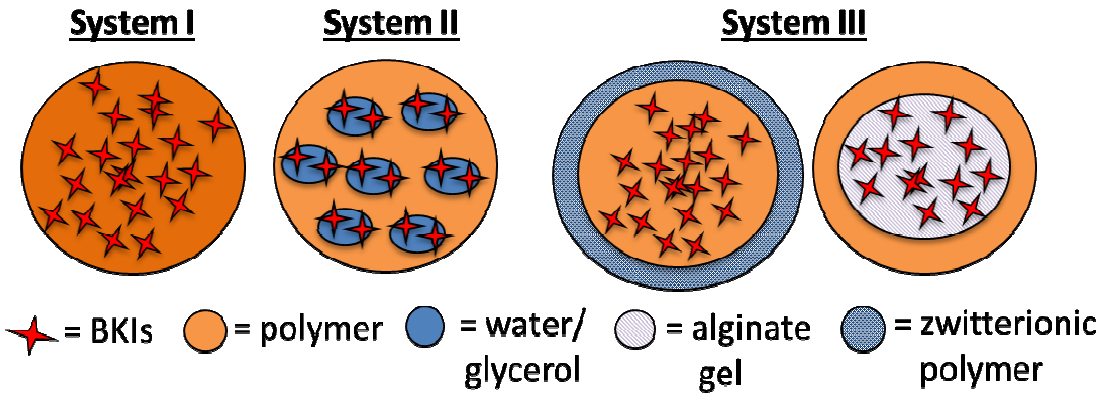

Figure 1: Schematic of the three particle systems for amphiphilic incorporation

BKI-loaded particles were characterized by size (via DLS and SEM), morphology, zeta potential, loading efficiency, and release of active BKI. System I, a basic emulsion system allows the amphiphilic BKI to be dispersed throughout the particle with BKI and PLGA polymer in the same phase. We altered the hydrophobicity of the single emulsion solution by altering the chemical composition with small additions of DMSO: water to the organic DCM. The average particle size was below 5um with a zeta potential close to zero as measured with DLS. Loading efficiency ranged from 0.9 – 1.6%.Results are given in Table 1.

Table 1: Characteristics of System I Particles.

PLGA	Zwt/Alg	DMSO	water	Avg. Size	Avg. Zeta Potential	BKI Loading Efficiency	Total Release (over 4 days)
(mg/mL)	(z/a)	(uL)	(uL)	(nm or um)	(Z)	(%)	(% mass)
50	---	0	0	1 – 5um	-0.2	0.9%	32
50	---	1	0	5 - 10um	-1.6	3%	5
50	---	1	1	5 - 10um	-1.9	4%	4
50	---	5	5	1 – 5um	-0.3	1.1%	25
50	---	2	98	1 – 5um	-0.2	0.4%	56
50	---	10	90	1 – 5um	-0.6	1.0%	31
50	---	10	90	790 - 940nm	-0.5	1.6%	76

System II double emulsion particles were fabricated with BKIs in either water or glycerol hydrophilic phase while the PLGA polymer was in the hydrophobic DCM phase. Emulsification via point sonication allowed the BKI to internalize into scattered hydrophilic pockets within the particle. Particles were in the nano and micro-sized ranges as per SEM. Zeta potential could not be measured due to majority of micron-sized particles. Loading efficiency improved to up to 5% as compared to the System I particles. Results are given in Table 2.

Table 2: Characteristics of System II Particles

PLGA	Zwt/Alg	DMSO	water	Avg. Size	Avg. Zeta Potential	BKI Loading Efficiency	Total Release (over 4 days)
(mg/mL)	(z/a)	(uL)	(uL)	(nm or um)	(Z)	(%)	(% mass)
50	---	5	0	200nm-30um	---	2%	6
50	---	5	5 (W)	30- 60um	---	5%	4
50	---	5	20 (W)	20-300um	---	4%	6
50	---	5	45 (W)	1 - 20um	---	4%	6
50	---	5	5 (G)	20 -90um	---	3%	10
50	---	5	20 (G)	100nm -30um	---	3%	14
50	---	5	45 (G)	50 – 200um	---	3%	12

System III, core/shell particles either consisted of a zwitterionic-shell or a hydrophilic gel-core. The zwitterionic polymer co-blocked with the PLGA polymer acting as an amphiphilic membrane for controlled release with the zwitterionic hydrophilic section directed to the surface of the particle, similar to a surfactant. Alternatively, the hydrophilic gel core particles had an alginate inner core with a hydrophobic PLGA outer shell. The inner gel core should facilitate the internalization of BKIs at the interphase for increased loading efficiency.

Zwitterionic co-blocked particles displayed an average particle size of 400nm to 5um with positive zeta potentials indicative of a successful co-block with the hydrophilic section of the zwitterionic polymer on the outer shell of the particle. Loading efficiency was below 1%.Hydrophilic alginate core particles displayed particle size between 2 – 10um with low loading incorporation of below 1%.Results are given in Table 3.

Table 3: Characteristics of System III Particles.

PLGA	Zwt/Alg	DMSO	water	Avg. Size	Avg. Zeta Potential	BKI Loading Efficiency	Total Release (over 4 days)
(mg/mL)	**(z/a)**	**(uL)**	**(uL)**	**(nm or um)**	**(Z)**	**(%)**	**(% mass)**
50	Zwit	1	0	1 - 5um	26	0.5%	25
50	Zwit	1	1	1 – 5um	29	0.5%	26
50*	Zwit	1	0	448 - 460nm	12	0.2%	all
50*	Zwit	1	1	458-462nm	17	0.2%	all
50	Zwit	5	5	750-810nm	27	0.1%	all
50	Zwit	10	90	535-550nm	23	0.1%	all
50	Zwit	10	90	400 - 410nm	9	0.04%	all
50	Alg	0.12	50	5 – 10um	-4	0.04%	all
50	Alg	0.24	100	2 – 5um	-23	0.1%	all

The slight increase in hydrophilicity due to increased DMSO: water additions in System I particles resulted in similar loading efficiency, but displayed initial burst of the particles with release tapering off by four days. The particles without DMSO: water mixed into DCM displayed a controlled release of BKI for over four days with no initial burst detected. System II particles all exhibited burst release regardless of internal phase chosen. Release tapered off by four days. System III zwitterionic shell particles exhibited two release profiles based on the particle size.

Nano-sized particles released all contents within four days, while the micron-sized particles released active BKI in a controlled manner for more than four days. System III hydrophilic alginate core particles had a very high initial burst releasing most of the incorporated BKI molecule within the first four hours. Results are given in Figure 2.

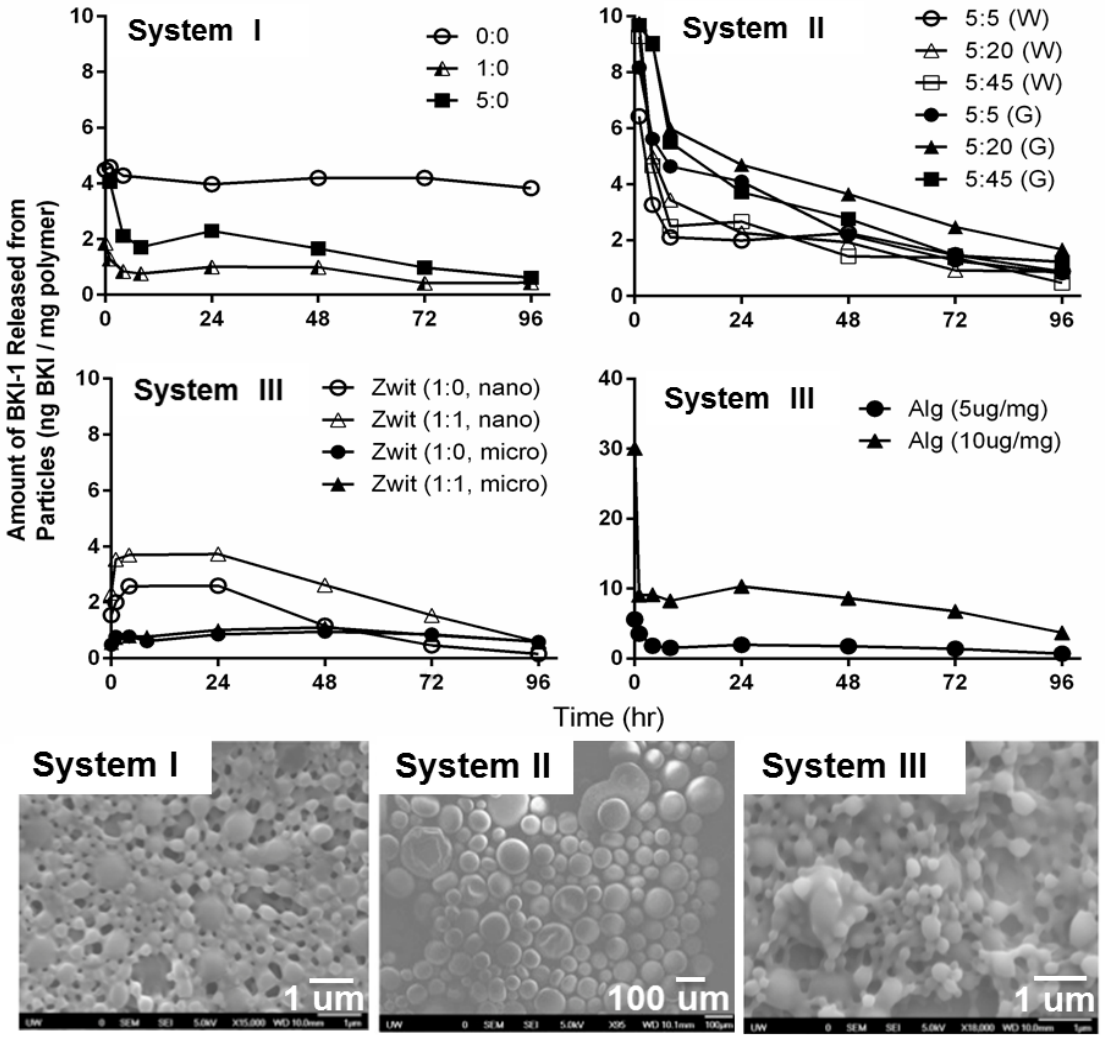

Figure 2: Release of BKI from particles in System I – III Ratios are DMSO:water unless otherwise indicated.
W = water, G = glycerol, Z_{wit} = zwitterionic shell particles, Alg = alginate core particles, nano = nano-sized particles, micro = micron-sized particles.

System I single emulsion particles without addition of DMSO: water were considered the best system to optimize further based on low particle size and controlled release profile for four days. System II particles were too large (micron-range) and exhibited burst release. Although System III zwitterionic-shell particles displayed a controlled release profile over four days for the micron-sized particles, these particles were larger than desired for the BKI administration discussed in this paper. However, this system is recommended for alternative amphiphilic molecules whose applications are not hindered by micron-sized particles. System III hydrophilic alginate core particles displayed a high initial burst with all of the incorporated BKI released by day 4.We suspect that the inner core swelled and released most of its contents within 4 hours. This would need to be stabilized prior to future amphiphilic molecule incorporation.

Optimization of the Amphiphilic BKI Partitioning Internally in Particles Improves Loading Efficiency.

We hypothesized that by raising the pH to greater than the pKa of BKIs, the proton on the nitrogens would be deprotonated, which would permit higher interaction, and thus incorporation with the polymeric/organic phase. This should enhance the interaction between the incorporated inhibitor and the hydrophobic polymer on a molecular scale without adverse effects on macro-scale particle characteristics. Additionally, 1294 has a marginally higher hydrophobicity caused by the affixed methyl group on the *piperidine* ring.

Significant properties of both BKI-1 and 1294 such as molecular weight and structure remain consistent making this an ideal situation to see effects of slight variations in amphiphilic molecules. Log P transitions of both inhibitors confirmed the shift of the amphiphilic BKIs from slightly hydrophilic to hydrophobic when pH was raised above BKIs' intrinsic pKa.

We changed pH of aqueous solutions used at various stages of fabrication processes of the non-fluidic process to enhance the retention of BKIs in polymeric phases. The pH was raised from 6.4 to 12 for the internal phase pH (for system II double emulsion systems), the fabrication pH which is the external continuous phase in emulsions, and the recovery pH during centrifugation and washing steps after particle formation. We used a pH of 12 to ensure that the partitioning of the BKIs was stable throughout the fabrication process.

Interestingly, the highest incorporation efficiency at above 90% was achieved when only the fabrication pH was changed to a basic environment of pH 12 and the recovery phase remained at pH of 6.4.This increase in fabrication pH optimized the partitioning of amphiphilic BKI further into the particles for enhanced stability. The BKIs are not partitioning into the continuous aqueous phase or remaining at the surface of the particles where most effects of burst release can be seen. This partition-optimized system was utilized in all subsequent experiments. Results are given in Figure 3.

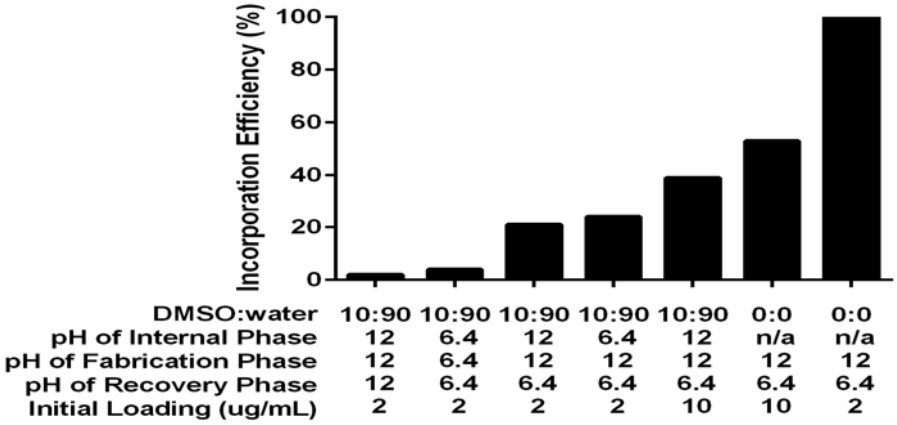

Figure 3: Incorporation efficiency for different pH conditions.

Partition-optimized Single Emulsion Particle Fabrication Improved Release Rates for Multiple Amphiphilic Molecules

Longer release studies were conducted by measuring the release of active BKI-1 or 1294 from the biodegradable partition-optimized PLGA particles for up to 4 weeks. Cumulative percent release was calculated based on the final loading of inhibitor incorporated into the particle. Both traditional non-fluidic (experiments above) and fluidic methods of fabricating partition-optimized particles were examined.

Average particle size was slightly larger for the fluidic method, although loading efficiency remained above 90% for all the loadings tested in both fluidic and non-fluidic partition-optimized particle systems. The BKI-1 displayed sustained release for the non-fluidic emulsion method, while the 1294-loaded particles displayed sustained release for all systems examined. Interestingly, the marginally higher hydrophobicity caused by the affixed methyl group on the *piperidine* ring of the 1294 amphiphilic inhibitor caused a definite increase in the sustained release profiles as compared to the BKI-1 amphiphilic inhibitor. Results are given in Figure 4.

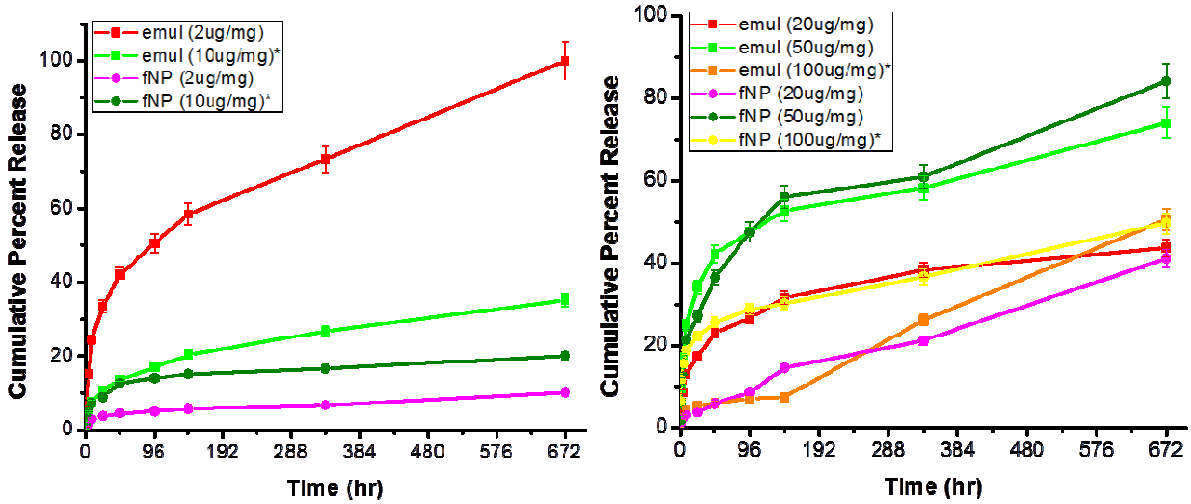

Figure 4: Cumulative percent release of BKI-1-loaded particles (left) and 1294-loaded particles (right) emul = emulsion/non-fluidic system, fNP= fluidic system

To finalize whether the non-fluidic or fluidic methods of partition-optimized particle fabrication would be better, we investigated the internal distribution of incorporated molecules via transmission electron microscopy (TEM).Using quantum dots, we were able to determine that the non-fluidic emulsion based system had a more uniform internal distribution of incorporated material compared to the fluidic system. The fluidic system displayed clustering of incorporated material which could lead to unsteady release of drugs. Results are given in Figure 5.

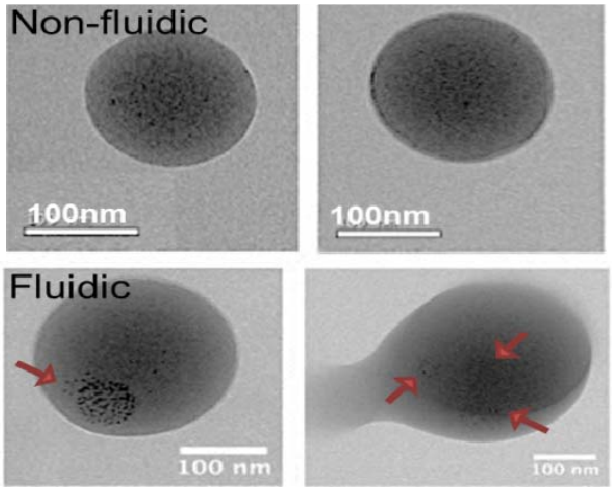

Figure 5: Comparison of the distribution of drugs in emulsion non-fluidic particles (top) and fluidic particles (bottom) Quantum dots were used for TEM visualization, arrows indicate clusters.

In addition, the BKI release studies displayed ideal sustained release for the emulsion-based non-fluidic method. Both amphiphilic BKIs provided a prolonged release for approximately 4 weeks and preliminary data suggests that the release rate should be above compound clearance levels. Therefore we recommend that the partition-optimized single emulsion particle fabrication system is the ideal choice for amphiphilic BKIs.

CONCLUSION

This study describes a novel approach to addressing an important public health challenge. The research work capitalizes on the unique chemical properties of BKIs to find a formulation that will deliver a steady blood concentration of the drug needed to effectively block transmission of malaria. This was achieved thorough analysis of fabrication systems for amphiphilic incorporation and optimization of the sustained release of two amphiphilic malaria transmission-blocking inhibitors to maintain a compound blood level above exflagellation EC50 for an extended period after single administration (Ojo KK et al, 2012). We are able to increase the incorporation of BKIs in polymeric particles to 90% by enhancing the partition of BKIs in polymeric organic phase with the partition-optimized method. Additionally, this further optimized the delivery system to provide a sustained release of these amphiphilic BKIs for up to 28 days, 40 times longer than the soluble half-life, and without the initial burst profile seen with similar fabricated particles. Further work to define the systemic application of this study in mouse model of malaria is currently being planned. Since these particles are within range for macrophage and innate immune cell phagocytosis, a parallel study is ongoing on modifying the surface of these PLGA particles to allow prolonged retainability in the tissue site as well as controlling overall immune system clearance while keeping the loading efficiency and release rates the same. Overall, this study was aimed at developing a delivery system for BKIs, but this extensive investigation of a range of fabrication systems, adaptability to slightly different amphiphilic BKIs, and partitioning of BKIs between organic and aqueous phases provides a base system for the sustained release of amphiphilic drugs.

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REFERENCES

Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK. (2011). Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. Malar J 10: 144.

Baker DA (2010). Malaria gametocytogenesis. Mol Biochem Parasitol 172: 57-65.

Bilati U, Allémann E, Doelker E (2003). Sonication parameters for the preparation of biodegradable nanocapsules of controlled size by the double emulsion method. Pharm Dev Technol 8: 1-9.

Bousema T, Drakeley C (2011). Epidemiology and infectivity of Plasmodium falciparum and Plasmodium vivax gametocytes in relation to malaria control and elimination. Clin Micro biol Rev 24: 377-410.

Cohen-Sela E, Teitlboim S, Chorny M, Koroukhov N, Danenberg HD. (2009). Single and double emulsion manufacturing techniques of an amphiphilic drug in PLGA nanoparticles: formulations of mithramycin and bioactivity. J Pharm Sci 98: 1452-1462.

Comm JE (2002). Annex v: Testing methods. A8 Partition Coefficient: Official Journal of the European Communities

Greenwood BM (2008). Control to elimination: implications for malaria research. Trends Parasitol 24: 449-454.

Igartua M, Hernández RM, Esquisabel A, Gascon AR, Calvo MB. (1997). Influence of formulation variables on the in-vitro release of albumin from biodegradable microparticulate systems. J Micro encapsul 14: 349-356.

Johnson SM, Murphy RC, Geiger JA, DeRocher AE, Zhang ZS. (2012). Development of toxoplasma gondii calcium-dependent protein kinase 1 (tgcdpk1) inhibitors with potent anti-toxoplasma activity. Journal of medicinal chemistry. 55: 2416-2426.

Ojo KK, Eastman RT, Vidadala R, Zhang Z, Rivas KL. (2014). A specific inhibitor of PfCDPK4 blocks malaria transmission: chemical-genetic validation. J Infect Dis 209: 275-284.

Ojo KK, Larson ET, Keyloun KR, Castaneda LJ, DeRocher AE. (2010). Toxoplasma gondii calcium-dependent protein kinase 1 is a target for selective kinase inhibitors. Nat Struct & Mol Biol 17:602-607.

Ojo KK, Pfander C, Mueller NR, Burstroem C, Larson ET. (2012). Transmission of malaria to mosquitoes blocked by bumped kinase inhibitors. J Clin Invest 122: 2301-2305.

Vidadala RS, Ojo KK, Johnson SM, Zhang Z, Leonard SE. (2014). Development of potent and selective Plasmodium falciparum calcium-dependent protein kinase 4 (PfCDPK4) inhibitors that block the transmission of malaria to mosquitoes. Eur J Med Chem 74: 562-573.

World Health Organization WHO (2014). World malaria report. Publications of the World Health Organization, Geneva Switzerland, pp:1-227.

Xie H, Smith JW (2010). Fabrication of PLGA nanoparticles with a fluidic nanoprecipitation system. J Nanobiotechnology 8: 18.

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