

Novel cellulose-based amorphous solid dispersions enhance quercetin solution concentrations *in vitro*

Andrew D. Gilley^a, Hale Cigdem Arca^b, Brittany L.B. Nichols^d, Laura I. Mosquera-Giraldo^e, Lynne S. Taylor^e, Kevin J. Edgar^{b,c}, Andrew P. Neilson^{a,*}

^a Department of Food Science and Technology, Virginia Tech, Blacksburg, VA 24060, United States

^b Macromolecules Innovation Institute, Virginia Tech, United States

^c Department of Sustainable Biomaterials, Virginia Tech, United States

^d Department of Chemistry, Virginia Tech, United States

^e Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette 47907, United States

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ABSTRACT

Quercetin (Q) is a bioactive flavonol with potential to benefit human health. However, Q bioavailability is relatively low, due to its poor aqueous solubility and extensive phase-II metabolism. Strategies to increase solution concentrations in the small intestinal lumen have the potential to substantially increase Q bioavailability, and by extension, efficacy. We aimed to achieve this by incorporating Q into amorphous solid dispersions (ASDs) with cellulose derivatives. Q was dispersed in matrices of cellulose esters including 6-carboxycellulose acetate butyrate (CCAB), hydroxypropylmethylcellulose acetate succinate (HPMCAS) and cellulose acetate suberate (CASub) to afford ASDs that provided stability against crystallization, and pH-triggered release. Blends of CASub and CCAB with the hydrophilic polyvinylpyrrolidone (PVP) further enhanced dissolution. The ASD 10% Q:20% PVP:70% CASub most significantly enhanced Q solution concentration under intestinal pH conditions, increasing area under the concentration/time curve (AUC) 18-fold compared to Q alone. This novel ASD method promises to enhance Q bioavailability *in vivo*.

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1. Introduction

Q (Fig. 1) is a dietary flavonol (a subclass of flavonoids) present at high levels in foods including apples, onions, and broccoli (Caridi et al., 2007; Price, Casuscelli, Colquhoun, & Rhodes, 1998). Q intake has been associated with many potential health benefits, including reduced risk of cardiovascular disease (Russo, Spagnuolo, Tedesco, Bilotto, & Russo, 2012), cancer (Firdous et al., 2014; Jaganathan, 2011; Zhou et al., 2010), and diabetes and obesity (Chiş, Baltaru,

Maier, Mureşan, & Clichici, 2013; Gutierrez, Prater, & Holladay, 2014; Song, 2005).

Poor Q oral bioavailability severely limits its potential to benefit health. This low bioavailability is largely due to its crystallinity, and hence poor solubility (ranging from 2.15 to 7.7 µg/mL at 25 °C (Lauro et al., 2002; Srinivas, King, Howard, & Monrad, 2010)) in the aqueous milieu of the gut lumen, as well as extensive metabolism and subsequent luminal efflux by gut epithelial cells (Phase-II and Phase-III xenobiotic metabolism, respectively). Improved Q solubility may increase bioavailability by increasing the amount available for absorption, and by saturating Phase-II and Phase-III metabolic enzymes; both effects are likely to result in increased net flux into circulation.

Many techniques have been employed to improve Q oral bioavailability, such as protein- or cellulose-based nanoparticles (Fang et al., 2011; Kakran, Sahoo, Li, & Judeh, 2012; Sahu, Saraf, Kaur, & Saraf, 2013), encapsulation (Dian et al., 2014), nanoemulsi-

Abbreviations: ASD, amorphous solid dispersion; CCAB, carboxycellulose acetate butyrate; CASub, cellulose acetate suberate; EC, epicatechin; HPMCAS, hydroxypropylmethyl cellulose acetate succinate; PVP, polyvinylpyrrolidone.

* Corresponding author at: Dept. of Food Science and Technology, Virginia Tech, 1981 Kraft Dr, Blacksburg, VA 24060, United States.

E-mail address: andrewn@vt.edu (A.P. Neilson).

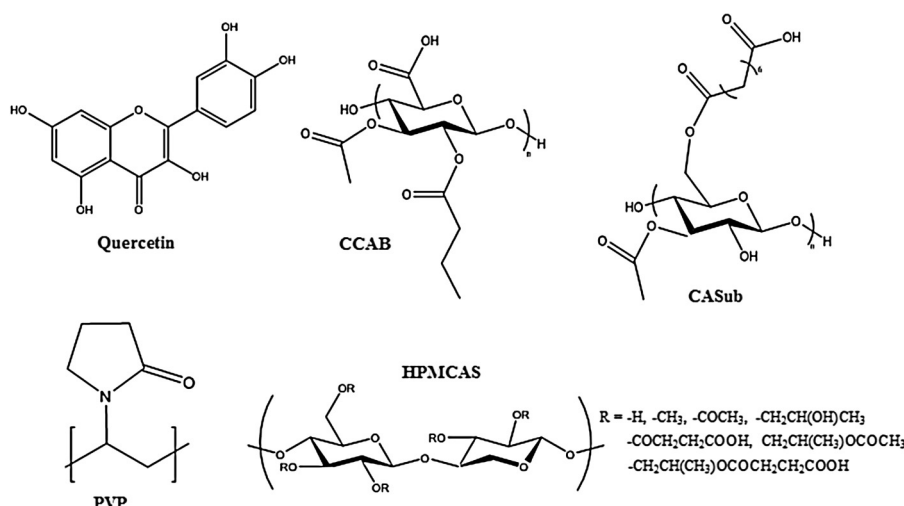


Fig. 1. Chemical structures of Q, CCAB, CASub, PVP, and HPMCAS. The cellulosic structures are not meant to convey regioselective substitution; depictions of substituent location are merely for convenience and clarity of depiction.

fying drug delivery systems (Tran, Guo, Song, & Bruno, 2016), and ASD (Ilevbare, Liu, Edgar, & Taylor, 2012; Li et al., 2013; Pereira et al., 2013). ASD preparation with polymer dispersants is an attractive way to stabilize the high energy, amorphous drug in a glassy polymeric matrix. Exposure of the ASD to the GI lumen not only provides supersaturated drug solutions, but also enhances permeation by increasing the drug concentration gradient across the enterocytes. Polymer selection is key for ASD performance because the dispersion must be miscible, with strong polymer–drug interactions (e.g. hydrogen bonding) for stability against crystallization (Pereira et al., 2013; Vasconcelos, Sarmiento, & Costa, 2007). Amphiphilic polymers possessing carboxylic acid functionality perform well in ASD due to strong polymer–drug interactions; their pH responsiveness is also valuable. At gastric pH, the protonated form protects the drug and minimizes release, while deprotonation at near-neutral intestinal pH swells the polymer and triggers drug release (Li et al., 2013; Pereira et al., 2013). Cellulose derivatives are popular ASD polymers due to their generally benign nature and high T_g values. CASub and cellulose acetate adipate propionate (CAAdP) were synthesized in the Edgar lab and show high promise for ASD (Li et al., 2013; Liu et al., 2014). Novel ASD polymers are needed to go beyond the performance of currently used polymers like PVP and HPMCAS that were not designed for ASD, and cellulosic polymers are ideal candidates due to their generally low toxicity and lack of oral bioavailability.

ASD has been only lightly explored for Q dissolution enhancement. Lauro et al. achieved slight dissolution enhancement using ASDs prepared with cross-linked sodium carboxymethylcellulose and sodium starch glycolate (Lauro et al., 2002). Similarly, Lauro et al. used spray dried dispersions with cellulose acetate trimellitate and cellulose acetate phthalate to improve Q release at pH 6.8 (Lauro, Maggi, Conte, De Simone, & Aquino, 2005). Recently, several polymers were evaluated for their ability to improve Q dissolution *in vitro* (Li et al., 2013). HPMCAS afforded ASDs containing up to 50% Q content; enhanced dissolution was obtained from 10% Q ASDs, optimally in polymer blends containing 10% of the water-soluble (PVP) (Gupta, Kakumanu, & Bansal, 2004; Konno, Handa, Alonzo, & Taylor, 2008; Van den Mooter et al., 2001). Employing PVP in blends with other cellulosic polymers may generally enhance drug release, while retaining the excellent stabilization from the cellulosic polymer (Marks, Wegiel, Taylor, & Edgar, 2014).

The objective of this study was to assess the performance of the novel polymer CASub for making Q ASDs and creating supersaturated Q solutions at physiological pH, vs. crystalline Q as negative

control and HPMCAS/Q ASD as positive control. We hypothesized that 1) CASub would provide enhanced solution concentration and preferable dissolution kinetics compared to HPMCAS, and 2) that blending CASub with PVP would further enhance Q dissolution.

2. Experimental

2.1. Materials

Quercetin ($\geq 95\%$ by HPLC), epicatechin (EC) ($\geq 90\%$ by HPLC), and KCl (solid, anhydrous, $\geq 99\%$) were purchased from Sigma-Aldrich. Cellulose acetate propionate (CAP-504-0.2; degree of substitution (DS) (acetate) = 0.04, DS (propionate) = 2.09; $M_n = 15,000$); CCAB; DS (butyrate) = 1.62, DS (acetate) = 0.06, DS (carboxylic acid) = 0.28; $M_w = 252,000$ and cellulose acetate (CA 320S, DS (acetate) = 1.82) $M_n = 50,000$ were from Eastman Chemical Company. HPMCAS (wt%: methoxyl 20–24%, hydroxypropyl 5–9%, acetyl 5–9%, succinoyl 14–18%; $M_w = 18,000$) was from Shin-Etsu Chemical Co., Ltd. Chemical structures of ASD polymers used (HPMCAS, PVP, CCAB, and CASub) are provided in Fig. 1. Acetonitrile (ACN, HPLC-grade), methylene chloride (HPLC-grade), tetrahydrofuran (THF), reagent ethanol, sodium phosphate monobasic, and sodium hydroxide (NaOH) were purchased from Fisher Scientific and used as received. HCl (12.1 M) was obtained from Macron Chemicals. Suberic acid, adipic acid, methyl ethyl ketone (MEK), *p*-toluenesulfonic acid (PTSA), triethylamine (Et_3N), and oxalyl chloride were purchased from ACROS Organics. 1,3-Dimethyl-2-imidazolidinone (DMI) was purchased from ACROS Organics and dried over 4 Å molecular sieves. Water was purified by reverse osmosis and ion exchange using a Barnstead RO pure ST (Barnstead/Thermolyne) purification system. LCMS grade ACN, water and formic acid were obtained from VWR.

2.2. Synthesis of CASub

CASub was synthesized as previously reported (Liu et al., 2014). See Supplemental Information for full details.

2.3. Preparation of ASDs via spray drying

Supplemental Information contains a full description of preparation of ASDs containing Q. Our convention for naming treatments is to list the % polymer(s), with the remainder being Q. For example, 10% Q/90% CCAB is referred to as 90 CCAB in the text, figures

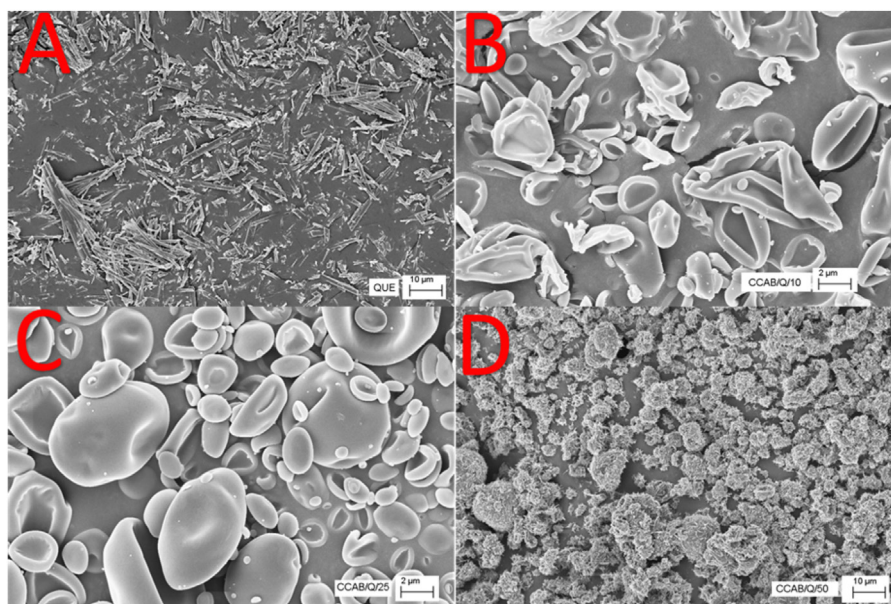


Fig. 2. SEM images (mag. 10 $\times$) for crystalline Q (A), 90 CCAB (B), 75 CCAB (C), and 50 CCAB (D) are shown to illustrate particle size range (1–3 μ m) and morphology.

and tables. ASDs prepared were: 90 CCAB, 75 CCAB, 50 CCAB, 10 PVP:80 CCAB, 20 PVP:70 CCAB, 90 HPMCAS, 90 CASub, 10 PVP:80 CASub, 20 PVP:70 CASub. ASD Q content was confirmed by UPLC to be within 4% of targeted value in every case (Supplementary Table 1).

2.4. ASD characterization

2.4.1 XRD, DSC and FT-IR

X-ray powder diffraction, DSC and FTIR were performed as described in in Supplementary information.

2.4.1. Determination of crystalline and amorphous Q solubility

2.4.1.1. Crystalline solubility. An excess of crystalline Q was added to 15 mL pH 6.8 buffer solution, or pH 2.5 solution. Solutions were equilibrated at 37 °C/48 h with constant agitation, protecting them from light.

2.4.1.2. Amorphous solubility. Supersaturated Q solutions were prepared by adding a specific amount of Q stock solution (4 mg/mL in MeOH) to 15 mL buffer at 37 °C. Potassium phosphate buffer (100 mM, pH 6.8) with 100 μ g/mL PVP (Kollidon® 12 PF); or acidified distilled water (pH 2.5) with 100 μ g/mL PVP was used. The polymer was added to inhibit drug crystallization during the experiment and accurately determine the “amorphous solubility” of Q. Total Q concentration of was 80 μ g/mL.

Crystalline and supersaturated solutions were centrifuged at 35,000 rpm (274,356 $\times$ g) for 30 min to separate the precipitated drug phase using an Optima L-100 XP ultracentrifuge equipped with Swinging-Bucket Rotor SW 41 Ti (Beckman Coulter, Inc., Brea, CA). Following centrifugation, the supernatant was collected, diluted (1:1) with methanol, and the final concentrations were measured by HPLC using an Agilent HPLC 1260 Infinity system (Agilent Technologies, CA, USA) with an Agilent Eclipse plus C18, 4.6 $\times$ 250 mm, 5 μ m analytical column (Agilent technologies, CA, USA). Full HPLC methodological details are presented in Supplementary information.

2.5. In vitro dissolution

In vitro dissolution was performed under non-sink conditions to evaluate Q dissolution concentrations and kinetics achieved via ASD delivery, compared to crystalline Q alone, under conditions similar to normal human gastrointestinal conditions. Full details in Supplemental Information.

2.6. UPLC–MS/MS

Internal standard solution (50 μ L, 0.8 mg/mL EC in ethanol), 50 μ L diluted dissolution supernatant, and 900 μ L of 0.1% formic acid in 80% water/20% 80:20 ACN/THF were added to Waters UPLC vials (Milford, MA) and mixed. Analysis of Q content within ASDs and subsequent *in vitro* dissolution solution concentrations were performed by UPLC–MS/MS (method details in Supplemental Information).

2.7. Data analysis and statistics

Dissolution results are reported as soluble Q vs. time. Dissolution kinetic parameters were determined from these data. Pseudo-pharmacokinetic parameters (area under the concentration-time curve: AUC; maximal observed solution concentration: C_{MAX} ; time at which maximal solution concentration was observed: T_{MAX}) were computed from Q concentration/time data using standard plugins for Microsoft Excel (Redmond, WA). Prism v. 6.0d (Graph-Pad, la Jolla, CA) was used to perform statistical comparisons. Dixon's Q-test (α = 0.05) was utilized to identify and exclude any outliers as necessary. Significant differences in dissolution parameters between treatments were determined using one-way ANOVA with Tukey's HSD *post hoc* test performed on treatment means. Significance was defined *a priori* as $P < 0.05$.

3. Results and discussion

Three promising carboxylated cellulose derivatives were selected for ASD preparation in order to increase Q apparent solubility (Fig. 1). CCAB is a new commercial carboxylated cellulose ester (Buchanan et al., 2014), and HPMCAS is a cellulose ether-ester that is in commercial use as an efficient ASD polymer (Li

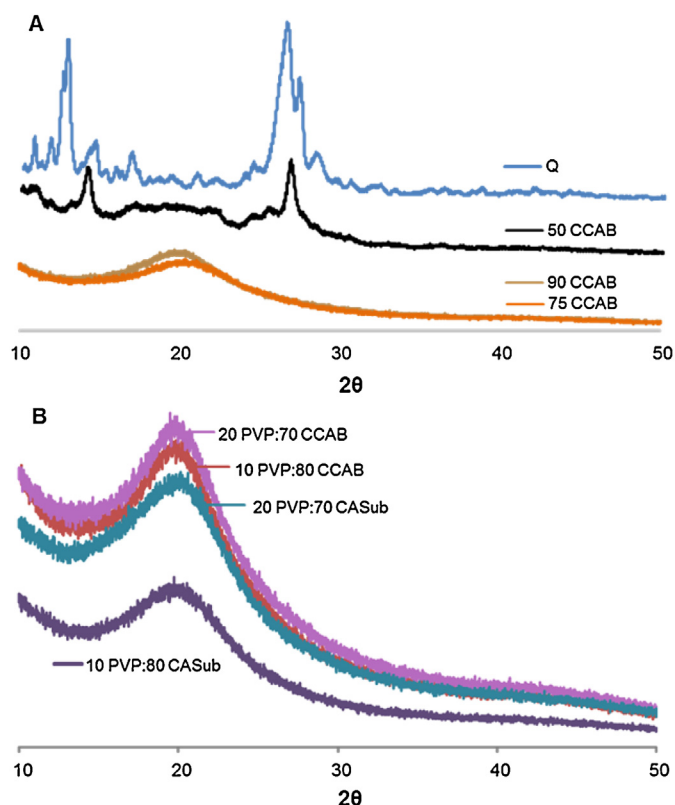


Fig. 3. XRD spectra of Q, 50 CCAB, 75 CCAB, and 90 CCAB (A) as well as the PVP blends with both CCAB and CASub (B).

et al., 2013). CASub was recently designed by the Edgar and Taylor groups as a promising ASD polymer and crystal growth inhibitor (Liu et al., 2014). The above-mentioned polymers may not only stabilize amorphous Q in the solid state and prevent recrystallization after release, but also provide targeted pH-controlled release to the small intestine where Q absorption occurs. We also investigated blends of CASub and CCAB with PVP to promote enhanced Q dissolution, since Q and thus its ASDs with polymers like CASub and CCAB ($\delta = 22.66$ and 24.44 , respectively) are rather hydrophobic. ASD drug concentration influences both the practicality of the method and ASD performance, so to explore this influence, Q concentration in the ASD was varied (10%, 25% and 50% Q(w/w)), using readily available commercial CCAB (90 CCAB, 75 CCAB, 50 CCAB) as the test system. To compare effectiveness of novel polymers and polymer blends, dissolution profiles were compared against commercial polymers HPMCAS and CCAB.

3.1. Solid state characterization of Q loaded ASDs

Morphology was examined by SEM (Fig. 2; other SEM images see Supplemental Fig. S1–4). All ASD particles exhibited a smooth surface, indicating absence of evident crystals, except for 50% Q loaded CCAB that appeared to contain Q crystals. Particles have corrugated morphology, crushed, indented and collapsed, typical of polysaccharide-based spray-dried ASDs.

XRD was used to determine whether dispersions were amorphous. All XRD spectra showed only amorphous haloes (Fig. 3), except those of crystalline Q and 50% Q in CCAB (Fig. 3A). XRD data strongly support the amorphous nature of these dispersions except for 50 CCAB, which was therefore excluded from further testing. XRD spectra for 90 HPMCAS and 90 CASub are presented in Supplemental Fig. 5.

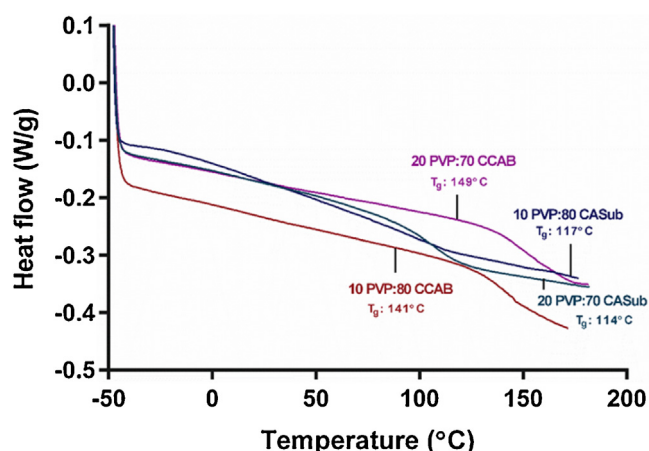


Fig. 4. DSC second heating curves of Q-loaded ASDs of CASub, CCAB, and their respective blends containing 20% PVP.

DSC was used to further examine dispersion morphology of Q and polymers; data from CASub and CCAB are presented in Fig. 4. Although crystalline Q melts at 326°C , DSC scans were kept $\leq 185^{\circ}\text{C}$, due to concerns about potential crosslinking of these polymers (containing both OH and CO_2H groups) above that temperature (Posey-Dowty et al., 2007; Shelton et al., 2009). For ASDs a glass transition (T_g) temperature lower than that of the pure polymer is expected. Polymer T_g values are 175°C (PVP), 144°C (CASub) and 134°C (CCAB). ASDs (10% Q) all had lower T_g values than the corresponding pure polymer, indicating that Q acted as a plasticizer. Since Q melts higher than the decomposition temperatures of several of our polymers, absence of T_m and T_c in the ASDs could not be confirmed. DSC T_g values along with the XRD data were sufficient to confirm the amorphous character of Q in these dispersions. DSC heating curves for all other dispersions are presented in Supplemental Fig. 6.

FT-IR spectra are presented and discussed in the Supplemental Information.

3.2. Determination of crystalline and amorphous Q solubility

We attempted to confirm the crystalline solubility and measure Q amorphous solubility (Table 1). Amorphous forms of compounds have a maximum apparent solubility, which represents the maximum amount of free drug achievable in solution, termed amorphous solubility (Ilevbare & Taylor, 2013). The experimental amorphous solubility can be measured by creating supersaturated solutions by a solvent-shift method, and then measuring the concentration of drug in the supernatant. However, for compounds that are fast crystallizers (like Q), it is necessary to add a small amount of polymer (in this case PVP) to stabilize the supersaturated solution, inhibiting crystallization, and permitting accurate measurement of amorphous solubility. It was impossible to measure Q amorphous solubility in the absence of polymer, because the drug crystallized upon contact with the aqueous solution. We measured Q crystalline solubility at low (2.5) pH, where Q is unionized, and at pH 6.8 where it is partially ionized. Q amorphous solubility was also measured at both pH values. Crystalline solubility values were determined in the absence and presence of PVP, while amorphous solubility was determined only in the presence of PVP. Amorphous solubility was significantly higher than its crystalline counterpart at both pH values. Q crystalline solubility was similar to literature reports, and amorphous solubility appears to be at least $31\text{ }\mu\text{g/mL}$ at small intestine pH.

Table 1
Crystalline and amorphous solubility of Q in varying dissolution medium (values are mean $\pm$ SEM).

Medium	Crystalline solubility ($\mu\text{g}/\text{mL}$)	Amorphous solubility ($\mu\text{g}/\text{mL}$)
Acidified water (pH 2.5) ^a	0.64 $\pm$ 0.11	N/A ^b
Acidified water (pH 2.5) ^a + 100 $\mu\text{g}/\text{mL}$ PVP	1.03 $\pm$ 0.20	23.48 $\pm$ 0.06
100 mM potassium phosphate buffer (pH 6.8)	1.03 $\pm$ 0.08	N/A ^b
100 mM potassium phosphate buffer (pH 6.8) + 100 $\mu\text{g}/\text{mL}$ PVP	N/A ^c	31.29 $\pm$ 1.80

^a Acidified with phosphoric acid.

^b Measurement not performed in absence of polymer due to fast Q crystallization upon contact with the solution.

^c Measurement not performed.

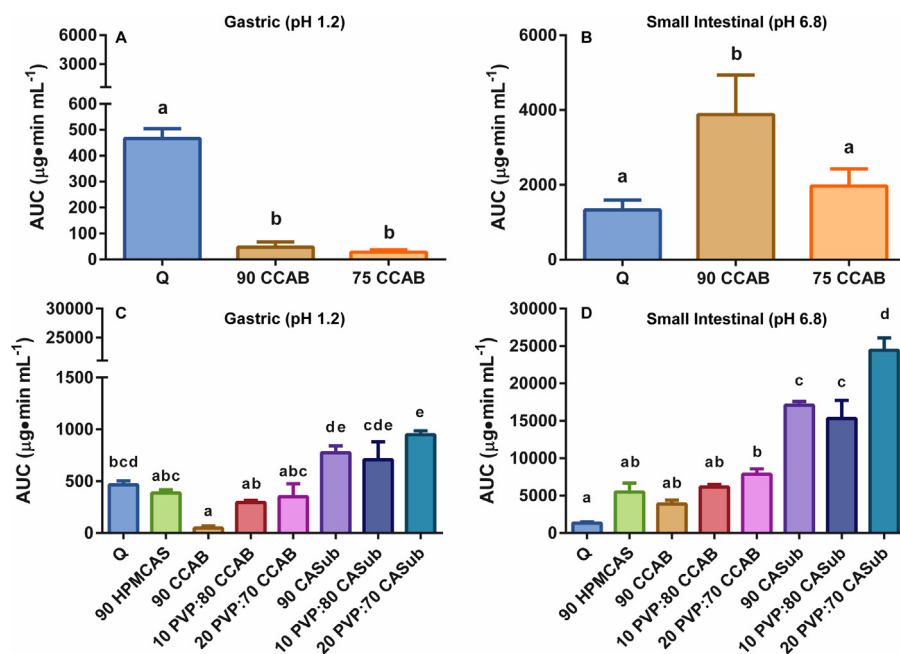


Fig. 5. Dissolution area under the curve (AUC) values for all treatments. Q compared to 90 CCAB and 75:25 CCAB is given for both gastric pH (A) and intestinal pH (B). Q compared to all treatments that employed 10% Q loads are shown for both gastric pH (C) and intestinal pH (D). Values are mean $\pm$ SEM. Different letters above each bar represent statistically significant differences in AUC between treatments ($p < 0.05$) by 1-way ANOVA with Tukey's post hoc test.

3.3. In vitro dissolution

3.3.1. Impact of Q content upon release

ASDs containing 10 and 25 wt% in CCAB were evaluated with regard to Q release. As presented in Fig. 5A, both 10% and 25% Q loaded CCAB ASDs (90 CCAB, 25 CCAB) effectively protected Q from release at acidic pH; indeed, solution concentrations were significantly lower than that from crystalline Q alone. At the neutral pH that mimics the small intestine, release from the 10% Q dispersion (90 CCAB, Fig. 5B) reached much higher solution concentrations than from the 25% Q ASD (75 CCAB) or from crystalline Q alone. This is predictable given the quite hydrophobic nature of Q (more Q in the ASD makes it more hydrophobic, slowing drug release), and is consistent with our results with CAAdP and other polymers (Li et al., 2013). As a result we chose to compare subsequent ASD formulations with different polymers using 10% Q in each ASD.

3.3.2. Simulated gastric conditions (pH = 1.2)

Crystalline Q and all Q ASDs had extremely low dissolution in the gastric environment (Fig. 5C, Supplemental Table 2). This was expected due to the inherent properties of crystalline Q, and of carboxylated cellulose derivatives (protonated, thus neutral/poorly soluble at acidic pH). Maximum low pH Q release was from 10% Q dispersed in 20% PVP:70% CASub, reaching only a mean C_{MAX} of 13.4 $\mu\text{g}/\text{mL}$ and AUC of 972 $\mu\text{g}\cdot\text{min}/\text{mL}$. All other polymers were relatively successful in preventing Q release at gastric pH. Even the

Table 2

Pseudo-pharmacokinetic parameters of Q at intestinal pH (6.8).

ASD Formulation	AUC ^a ($\mu\text{g}\cdot\text{min}/\text{mL}$)	C_{MAX} ^a ($\mu\text{g}/\text{mL}$)	T_{MAX} ^a (min)
Q	1330 $\pm$ 133	6.16 $\pm$ 0.141	120
90 CCAB	3880 $\pm$ 529	18.4 $\pm$ 2.15	60
75 CCAB	1940 $\pm$ 254	7.16 $\pm$ 1.49	60
50 CCAB	2550 $\pm$ 137	8.21 $\pm$ 0.760	N/A ^b
10 PVP:80 CCAB	6210 $\pm$ 382	20.0 $\pm$ 1.33	480
20 PVP:70 CCAB	7850 $\pm$ 733	27.4 $\pm$ 3.03	420
90 HPMCAS ^c	5480 $\pm$ 1210	64.1 $\pm$ 19.7	30
90 CASub ^c	17100 $\pm$ 485	48.7 $\pm$ 1.74	N/A ^b
10 PVP:80 CASub ^c	15300 $\pm$ 2434	50.4 $\pm$ 4.58	90
20 PVP:70 CASub ^c	24400 $\pm$ 1640	78.3 $\pm$ 7.12	N/A ^b

^a Data are mean $\pm$ SEM AUC, average C_{MAX} and T_{MAX} ($n = 4$ except where indicated).

^b T_{MAX} values listed as "N/A" had 4 separate times where C_{MAX} occurred.

^c $n = 3$.

AUC from 20%PVP:70% CASub at pH 1.2 was quite low compared to observed AUC values for any treatment at pH 6.8.

3.3.3. Simulated small intestinal conditions (pH = 6.8)

Dissolution experiments were performed at pH 6.8 to mimic the small intestine, and under non-sink conditions where ≥ 31 -fold supersaturation would be achieved if all drug dissolved, in order to permit observation and quantification of the supersaturation expected from ASDs. AUC measurements for all formulations are presented in Fig. 5D; AUC, C_{MAX} , and T_{MAX} values are summarized in Table 2. All ASDs examined provided some degree of

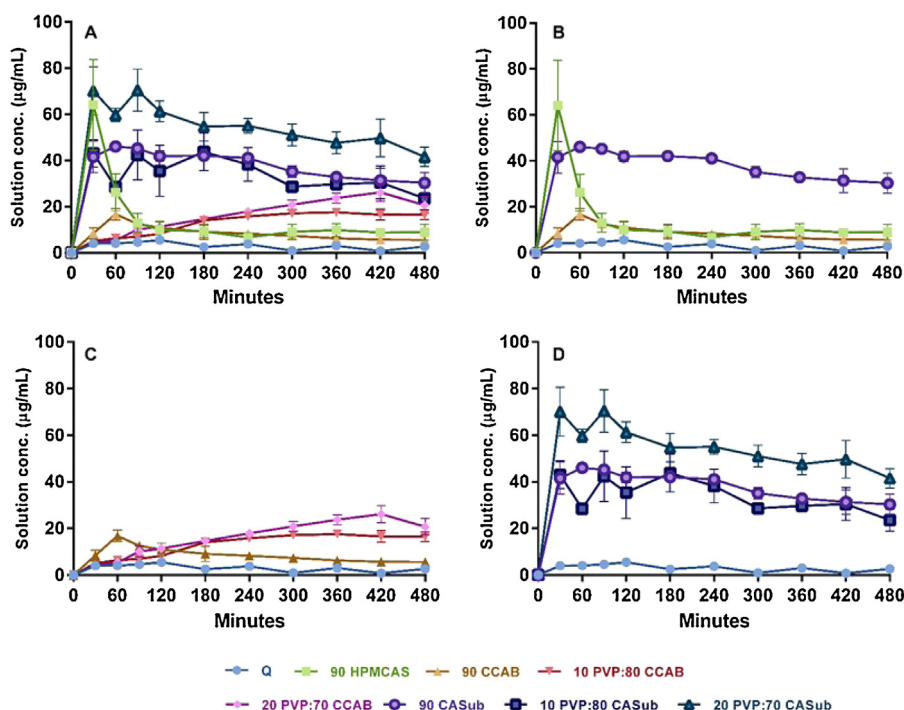


Fig. 6. Average solution concentrations of Q (mean $\pm$ SEM, $n=3-4$) plotted over time at pH 6.8 for all treatments (A), 10% Q loaded ASDs only shown for comparison of dissolution properties for each polymer utilized (B), the impact of PVP blending with CCAB (C) and CASub (D). All graphs contain crystalline Q as a control for comparison. Values are mean $\pm$ SEM.

supersaturation vs. Q alone. ASDs prepared with only HPMCAS or CCAB provided only modest (non-significant) improvements in Q solution concentrations; this is particularly interesting for HPMCAS, which has some solubility in water (ca. 23 mg/mL) (Li et al., 2013). In contrast, CCAB blended with PVP, and CASub either by itself or blended with PVP all gave very significantly supersaturated Q solutions. Optimum Q dissolution was observed with CASub and PVP:CASub blends. Overall, 10% Q dispersed in 20 PVP:70 CASub provided the most significant enhancement ($p < 0.05$) in Q apparent solution concentration, with an 18-fold increase in AUC compared to crystalline Q ($\sim 24,400$ vs. $1330 \mu\text{g min/mL}$, respectively). This ASD was also able to produce the highest maximum solution concentration ($C_{\text{MAX}} = 78.3 \mu\text{g/mL}$) over the course of 8 h at pH 6.8. This average C_{MAX} value is 12.7-fold higher than the average C_{MAX} value attained by crystalline Q ($6.16 \mu\text{g/mL}$). The degrees of enhancement of Q solution concentrations achieved are comparable to those achieved from different amorphous matrices by others (Fujimori et al., 2015) and in our previous work (Li et al., 2013).

Blending with the miscible and hydrophilic PVP enhanced Q release from both CCAB and CASub as anticipated. The concern was whether the lower concentration of the effective crystallization inhibitors CCAB ((Marks et al., 2014) manuscript in preparation) or CASub (Ilevbare, Liu, Edgar, & Taylor, 2013) would lead to loss of Q solution concentration due to crystallization. This does not appear to have been the case, and the blending approach was effective at synergistically combining PVP release properties with CASub/CCAB crystallization inhibition properties. Our data suggest that combination of hydrophobic (CCAB and CASub) and hydrophilic (PVP) polymers appears to provide relative protection from gastric conditions, enhanced release profile, and prevent Q recrystallization once in solution.

Polymer solubility parameters, presented in Table 3, are important, albeit imperfect, predictors of whether the polymer has the right hydrophobic/hydrophilic balance (they do not have the ability to discriminate between ionized and un-ionized carboxyl, which for this purpose is an important flaw). Solubility

Table 3

Hildebrand solubility parameters of polymers used to prepare Q ASDs.

Polymer	Solubility parameter, $\delta(\text{MPa}^{1/2})$
PVP	28.39
CCAB	24.44
CASub (DS 0.9)	22.66
HPMCAS	22.42

parameter calculations were performed as previously reported (Fedors, 1974; Liu et al., 2014) and can be found in Supplemental Information. Dissolution curves obtained with different ASDs are shown in Fig. 6 (pH 6.8) and Supplemental Fig. 8 (pH 1.2). Higher solubility parameters indicate greater polymer hydrophilicity, therefore the polymers arranged by decreasing hydrophobicity are PVP < CCAB < CASub < HPMCAS. The correlation between Q release and polymer solubility parameter is rather weak; this has been observed also in other polymer-drug systems (Mosquera-Giraldo, L.I., Meng, X., Edgar, K.J., Slipchenko, L.V., Taylor, L.S., manuscript under review). Within more confined data sets, solubility parameters can have predictive value; thus, addition of the quite hydrophilic PVP enhances release from the more hydrophobic CCAB and CASub matrices in predictable fashion. Based on solubility parameters alone, for example, the low Q solution concentration obtained from the 10% Q ASD in CCAB was unexpected; a maximum Q solution concentration of only $18.4 \mu\text{g/mL}$ (Fig. 6B) was attained. Lower concentration with CCAB than with CASub was surprising since CCAB has higher calculated solubility parameter than CASub. CASub was especially effective at enhancing Q solution concentration, reaching a maximum of $48.7 \mu\text{g/mL}$ within the first hour of dissolution (Fig. 6B). This is fully consistent with the known excellence of CASub as a crystallization inhibitor (Ilevbare et al., 2013). When blended with 10% PVP, release and thus solution concentration did not improve noticeably ($50.4 \mu\text{g/mL}$), but with 20% PVP in the ASD (20 PVP:70 CASub), solution concentration improved markedly to $78.3 \mu\text{g/mL}$ (Fig. 6D).

HPMCAS, the most hydrophobic polymer in the set of polymers investigated, provided interesting Q ASD behavior. Release from the HPMCAS ASD was very rapid, reaching an average solution concentration of 64.1 $\mu\text{g/mL}$ (Fig. 6B) within 30 min. This solution was clear, but quickly became cloudy, and measured solution concentration quickly dropped off, indicating that HPMCAS was ineffective at preventing Q crystallization from supersaturated solution.

3.4. Summary of results

Amorphous solid dispersions at 10 wt% Q were prepared using the cellulose esters CCAB, HPMCAS, and CASub. Q was also amorphous at 25% in CCAB, but was not fully amorphous at 50% Q in CCAB. All ASDs of Q in these carboxyl-containing cellulose esters protect effectively against Q release at fasting gastric pH, giving substantially lower Q concentrations than from Q without polymer. In contrast, all three cellulose esters provide supersaturated solutions of Q at small intestinal pH, at which pH most of the carboxylic acids are ionized. The degree of supersaturation from CCAB-only dispersions was slight, due in part to ineffective drug release, while release from HPMCAS ASD was rapid, achieving substantial supersaturation. However HPMCAS proved to be a poor inhibitor of Q recrystallization from supersaturated solutions, resulting in rapid de-supersaturation. CASub on the other hand provided both substantial Q release at pH 6.8, and stable, high supersaturation. Furthermore, incorporation of the more hydrophilic and water-soluble PVP into ASD blends was effective, enhancing release from CCAB at 10% PVP, and significantly enhancing release and supersaturation from CASub ASDs at 20% PVP. Thus CASub and its blends with PVP are highly effective polymers for enhancing Q solution concentration *in vitro*, and provide a promising opportunity for increasing Q bioaccessibility and bioavailability *in vivo*.

4. Conclusions

Overall these results confirm our hypotheses, and significantly illuminate structure-property relationships of ASD polymers. We can term polymer properties like sufficiently high T_g (50 °C or more above the highest likely ambient temperature) and solubility parameters within an effective range (hydrophobic enough to interact with hydrophobic drugs, hydrophilic enough to release them) as *necessary* but clearly not *sufficient* polymer properties for effectiveness in ASD. This work further confirms the value of the pH-responsive carboxylic acid functional group in providing neutral pH release as well as desirable specific polymer-drug interactions, but this parameter alone is also *insufficient* to fully predict success or failure. This work also provides a further example of the value of polymer blends for achieving performance levels (in this case both release and crystallization inhibition) that would be difficult to achieve by ASD of drug, in this case Q, with a single polymer. More detailed study of expanded sets of polymers is necessary to further sort out the structural features required for effective ASD.

The results of this study, particularly with CASub, warrant *in vivo* investigation of Q ASDs as method for increasing Q bioavailability upon oral administration. They predict that significant supersaturation should be achievable *in vivo*; it will be of great interest to see whether this results in higher permeation *in vivo*, and in saturation of metabolic enzymes, thereby providing enhanced bioavailability, particularly of the unmetabolized native Q. If successful, such an ASD approach should enable animal and human *in vivo* bioavailability enhancement studies, and provide predictable absorbed doses that will enable informative dose-response studies, thus leading to exploration of whether the potential health benefits of Q can be realized in humans.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.carbpol.2016.09.067>.

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