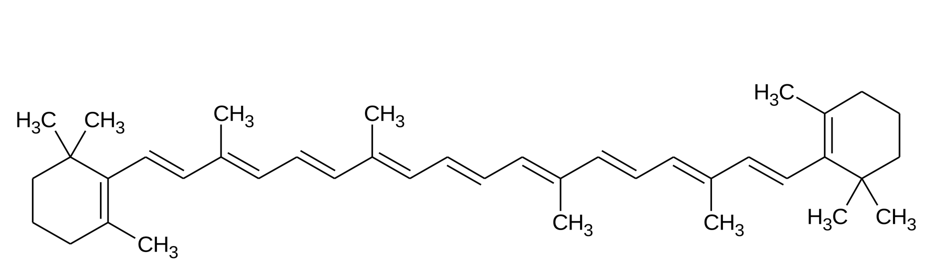
Co-encapsulation of β-Carotene and Quercetin in a nanoparticle using biodegradable polymers

Mira Buhecha*, Chunyu Shi*, Satyanarayana Somavarapu§, Ananth S V Pannala*

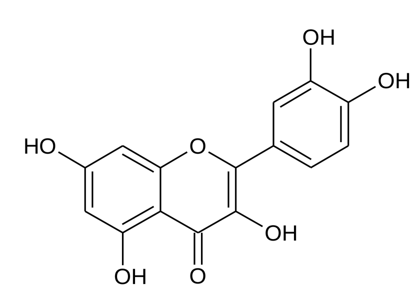
*School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton – BN2 4GJ, UK; §School of Pharmacy, University of London, London – WC1N 1AX, UK

INTRODUCTION

- β-Carotene (Bc) and Quercetin (Qu) are two naturally occurring antioxidants with a log P of 15 and 2, respectively.
- Poly(L-)Lactic acid (PLA) and Polylacticglycolic acid (PLGA) are biodegradable polymers that are known to be used for extended/sustained release of drugs.
- Encapsulation of molecules within nanoparticles had previously been demonstrated to increase the efficiency of drug targeting
- A co-encapsulated nanoparticle containing β-Carotene and Quercetin may demonstrate a synergistic antioxidant effect



β-Carotene



Quercetin

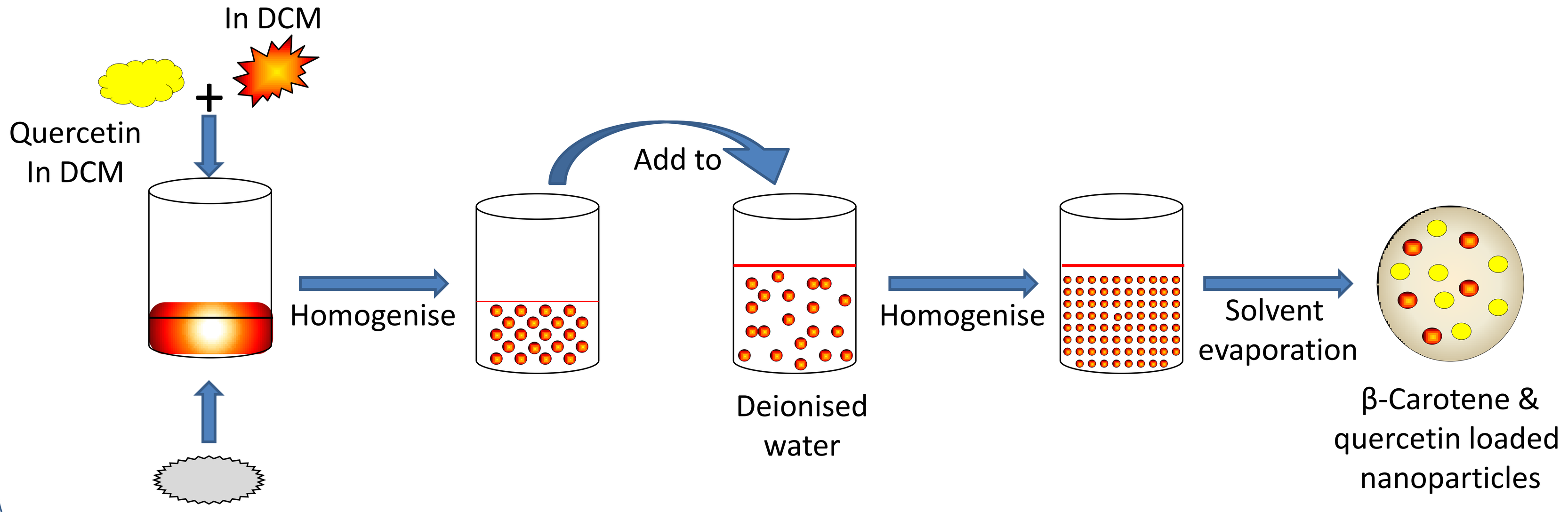
PURPOSE

The aim of the current study was

- To develop and characterize a co-encapsulated nanoparticle system consisting of quercetin and β-carotene using biodegradable polymers, PLA and PLGA
- To investigate the antioxidant activity of quercetin and β-carotene when incorporated in a single- and co-encapsulated nanoparticle system.

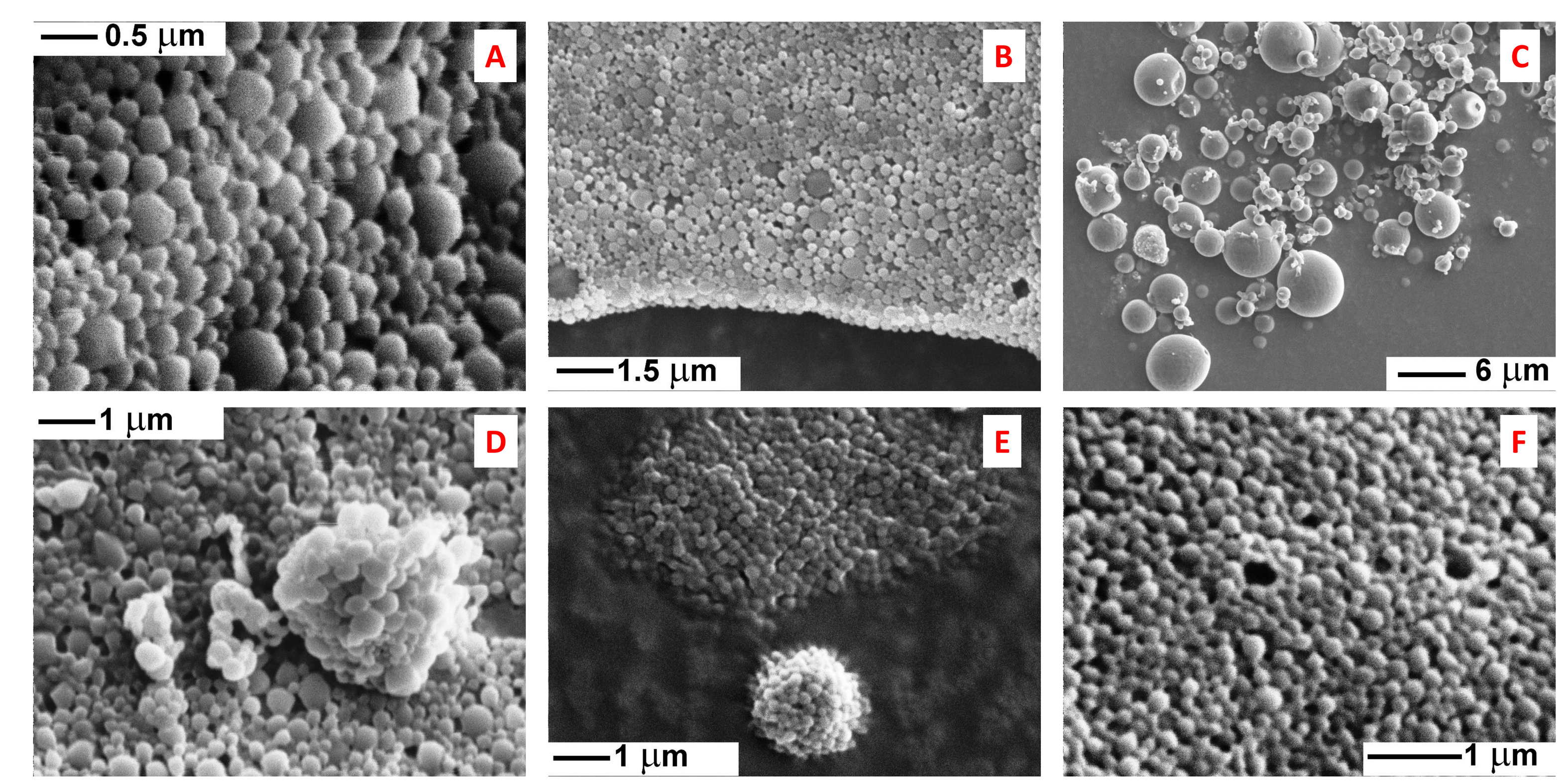
METHODS

- β-Carotene and quercetin were prepared (single and co-encapsulation) using a biodegradable polymer by emulsification/solvent evaporation technique
- A number of formulation approaches varying in drug/polymer concentration were investigated
- A known amount of the antioxidants (5 mg each) were encapsulated using either PLA, PLGA (75:25) or PLGA (50:50). PVA (115K) was used as the surfactant
- The nanoparticles were characterised for particle size and zeta-potential using the Zetasizer
- Loading efficiency of the nanoparticles was determined by selectively extracting quercetin into a mixture of acidified methanol (60%) and water (40%) followed by extracting β-Carotene into DCM. The samples were analysed by RP-HPLC
- FT-IR spectra were obtained to determine the presence of any non-encapsulated drug particles in the samples and Scanning electron microscopy (JEOL-JSM6310) was performed to examine the morphology and particle size distribution.
- Antioxidant activity was determined using the modified ABTS, (2,2'-azino bis (3-ethylbenzthiazoline-6-sulphonic acid)) radical cation scavenging assay where percentage reduction of absorbance of the radical cation was measured by spectrophotometry
- Antioxidant activity of blank, single and co-encapsulated nanoparticles was measured over a 24 hour period. Activity was compared using standard antioxidant solutions.



RESULTS

Figure 1: SEM images of nanoparticles (coated with palladium). A: PLA co-encapsulated, B: PLGA 75:25 co-encapsulated, C: PLGA50:50, D: PLA co-encapsulated, E: PLGA75:25 co-encapsulated, F: PLGA co-encapsulated



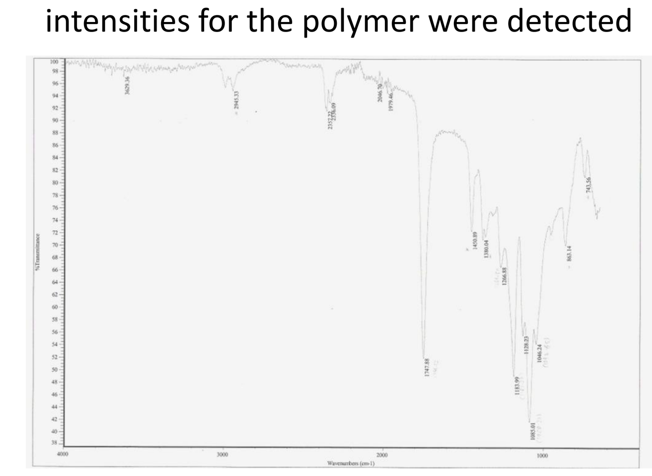


Figure 2: FT-IR spectra of PLA co-encapsulated sample. Only the bond intensities for the polymer were detected

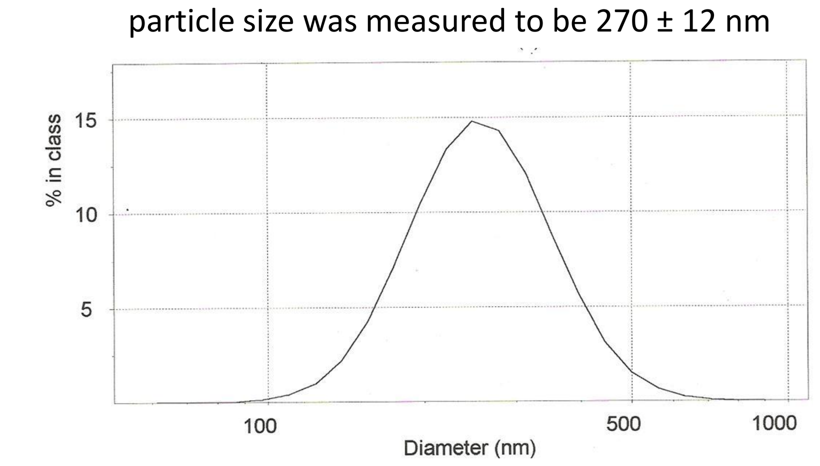


Figure 3: Size distribution of PLA co-encapsulated nanoparticle sample. Mean particle size was measured to be 270 ± 12 nm

Figure 4: Representative chromatogram of HPLC analysis of Quercetin (A [methanol: water:0.1N HCl 60:39.5:0.5] 60%: B [MeCN] 40%)

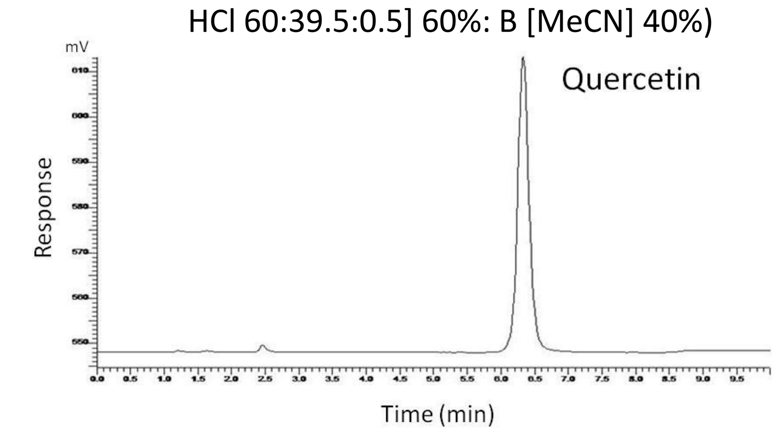


Figure 5: Representative chromatogram of HPLC analysis of β-carotene (methanol:DCM:MeCN 20:25:55)

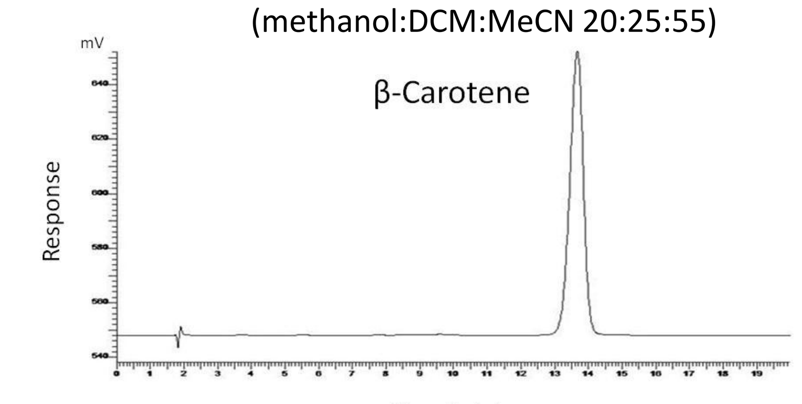


Table 1: Loading efficiency of the nanoparticles with the two antioxidants (n=3)

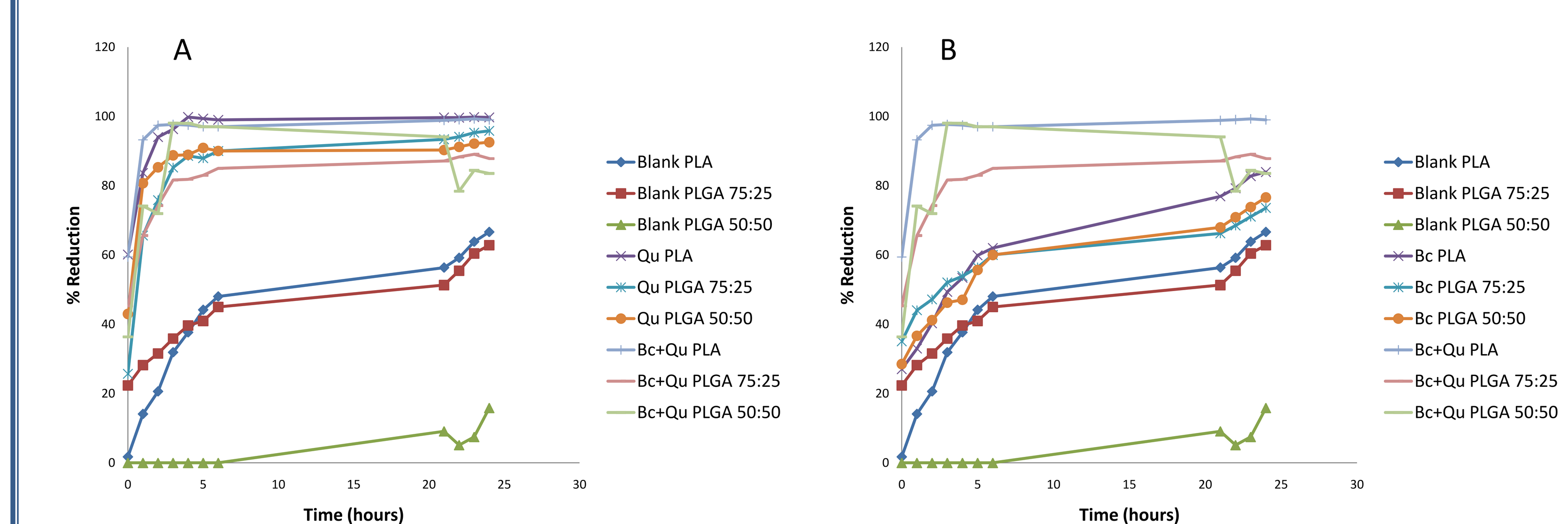
		Loading efficiency (%)	
Sample	Polymer	β-carotene	Quercetin
Single encapsulated β-carotene	PLGA 50:50	1.75 ± 0.09	-
Single encapsulated β-carotene	PLGA 75:25	0.86 ± 0.04	-
Single encapsulated β-carotene	PLA	0.34 ± 0.02	-
Single encapsulated Quercetin	PLGA 50:50	-	11.62 ± 0.58
Single encapsulated Quercetin	PLGA 75:25	-	25.44 ± 1.17
Single encapsulated Quercetin	PLA	-	33.6 ± 1.68
Co-encapsulated β-carotene and Quercetin	PLGA 50:50	1.49 ± 0.08	24.11 ± 1.21
Co-encapsulated β-carotene and Quercetin	PLGA 75:25	3.56 ± 0.18	28.25 ± 1.41
Co-encapsulated β-carotene and Quercetin	PLA	7.28 ± 0.36	49.30 ± 3.55

RESULTS

Table 2: Mean particle size and zeta potential of single-encapsulated and co-encapsulated nanoparticles (using either PLA, PLGA75:25 or PLGA 50:50) containing β-carotene and Quercetin

Sample	Polymer Used	Mean Size (nm)	Mean zeta potential
Blank	PLGA 50:50	284 ± 14	-0.8
Blank	PLGA 75:25	244 ± 12	-30.6
Blank	PLA	267 ± 13	-24.0
Single encapsulated β-Carotene	PLA	268 ± 13	-15.4
Single encapsulated β-Carotene	PLGA 50:50	229 ± 11	-10.2
Single encapsulated β-Carotene	PLGA 75:25	252 ± 13	-9.2
Single encapsulated Quercetin	PLA	263 ± 13	-26.6
Single encapsulated Quercetin	PLGA 50:50	281 ± 14	-22.2
Single encapsulated Quercetin	PLGA 75:25	264 ± 13	-7.7
Co-encapsulated β-carotene and Quercetin	PLGA 50:50	277 ± 14	-15.1
Co-encapsulated β-carotene and Quercetin	PLGA 75:25	282 ± 14	-41.6
Co-encapsulated β-carotene and Quercetin	PLA	270 ± 12	-35.4

Figure 6: Comparison of antioxidant activity of co-encapsulated nanoparticles with single encapsulated nanoparticles for Quercetin (A) and β-Carotene (B)



CONCLUSIONS

- Quercetin and β-carotene were successfully co-encapsulated in nanoparticles
- Quercetin was encapsulated to a greater extent than β-carotene
- However, significantly higher amount of β-carotene was encapsulated when co-encapsulated with quercetin than when single encapsulated. This observation was found to be true with all the polymers tested.
- Mean particle size for all the nanoparticles ranged between 240 to 300 nm
- The negative zeta potential observed indicates a stable suspension of the nanoparticles when dispersed in a solution
- Antioxidant activity of the compounds when encapsulated was observed to have a faster rate of reaction when co-encapsulated. Nanoparticles containing quercetin alone had a faster rate of reaction compared to β-carotene encapsulated nanoparticles.
- The observations made in this study can increase the potential to develop nanotechnology as a means of delivering a combination of antioxidants/drugs for various health benefits

REFERENCES

- Song X, Zhao Y, Wu W, Bi Y, Cai Z, Chen Q, Li Y and Hou S. PLGA nanoparticles simultaneously loaded with vincristine sulfate and verapamil hydrochloride: Systematic study of particle size and drug entrapment efficiency. *Int. J. Pharmaceutics*. **350**(1-2), 320-329, (2008)
- Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids Surf B Biointerfaces.* **75**(1), 1-18, (2009)
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C. Antioxidant Activity applying an improved ABTS Radical Cation Decolorization Assay. *Free Rad. Biol. Med*. **26**(9-10), 1231-1237, (1999).