



University of HUDDERSFIELD

University of Huddersfield Repository

Waters, Laura J., Swaine, Tanya and Lewis, Andrew

A calorimetric investigation of doxorubicin-polymer bead interactions

Original Citation

Waters, Laura J., Swaine, Tanya and Lewis, Andrew (2015) A calorimetric investigation of doxorubicin-polymer bead interactions. *International Journal of Pharmaceutics*, 493 (1-2). pp. 129-133. ISSN 0378-5173

This version is available at <http://eprints.hud.ac.uk/25351/>

The University Repository is a digital collection of the research output of the University, available on Open Access. Copyright and Moral Rights for the items on this site are retained by the individual author and/or other copyright owners. Users may access full items free of charge; copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational or not-for-profit purposes without prior permission or charge, provided:

- The authors, title and full bibliographic details is credited in any copy;
- A hyperlink and/or URL is included for the original metadata page; and
- The content is not changed in any way.

For more information, including our policy and submission procedure, please contact the Repository Team at: E.mailbox@hud.ac.uk.

<http://eprints.hud.ac.uk/>

1 **A calorimetric investigation of doxorubicin-polymer bead interactions.**

2

3 Laura J. Waters^{1*}, Tanya S. Swaine^{1,2}, Andrew L. Lewis²

4 ¹ Department of Pharmacy, School of Applied Science, University of Huddersfield, Queensgate, Huddersfield,
5 HD1 3DH, UK, l.waters@hud.ac.uk

6 ² Biocompatibles UK Ltd, a BTG International group company, Lakeview, Riverside Way, Watchmoor Park,
7 Camberley, Surrey, GU15 3YL

8 *Corresponding author. Tel: +44-1484-472190. E-mail address: l.waters@hud.ac.uk

9 Keywords: doxorubicin; ITC; thermodynamics; binding; DC Bead™; polymer

10

11 **Abstract**

12 Isothermal titration calorimetry (ITC) was utilised to investigate suitability of the technique to determine
13 the stoichiometry and thermodynamics of the interactions that occur between a commonly used chemotherapeutic
14 drug, namely doxorubicin, and a polymer bead-based drug delivery embolisation system (DC Bead™). Six
15 temperatures were selected for drug-polymer titrations (293 to 313 K) and in all cases an initially exothermic signal
16 reverted to an endothermic response upon the saturation of the beads with drug. From these experiments, and
17 subsequent calculations, the molar ratio of drug to SO₃⁻ (polymer) was found to be 0.4:1 at all temperatures studied.
18 Enthalpic data was calculated from the raw ITC data with an average enthalpy of drug-polymer binding of – 14.8
19 kJmol⁻¹ at 293 K through to – 19.4 kJmol⁻¹ at 313 K implying the process is enthalpically-driven yet only affected by
20 an increase in experimental temperature to a limited extent whereby an increase in experimental temperature results
21 in a small increase in the negativity in change in enthalpy recorded. The application of ITC in this study (with its
22 unique ability to monitor real-time interactions and facilitate stoichiometric calculations) resolves the lack of
23 knowledge regarding the thermodynamics of this specific drug-polymer interaction. This study confirms that ITC is
24 not only useful for this specific system, but also highlights the potential use of ITC for more general studies in this
25 area.

26

27 **Introduction**

28 Doxorubicin is a highly potent anticancer drug, widely used in chemotherapy for the treatment of various
29 types of cancers such as Kaposi's sarcoma, ovarian carcinoma and breast cancer (Rana et al., 2011). Despite a high
30 degree of anti-tumour activity, drug resistance and strong adverse effects limit its efficacy. To overcome this
31 problem several delivery strategies have been developed such as solid lipid nanoparticles and nanoliposomal
32 delivery systems, for example Doxil® (Natarajan et al., 2014). Ion-exchange microspheres and, in particular, drug-

33 eluting beads, have become the system of choice for drug delivery (Liu et al., 2001; Shuhendler et al., 2010). For
34 over a decade transarterial chemoembolisation using drug eluting beads (DEBs) has been a treatment option for
35 primary and secondary liver cancers. These drug/device combination microspheres are delivered intra-arterially into
36 tumour-feeding vessels to occlude blood flow, locally delivering a controlled and sustained therapeutic dose and
37 reduced systemic exposure of cytotoxic cancer drugs (Kerr, 1987). Interactions between the drug and these more
38 recently developed delivery systems is of interest for several reasons, primarily as it is important to fully
39 characterise such formulations to help optimise their bioavailability for the patient. However, the self-association of
40 the drug in aqueous solutions complicates the interpretation of the binding of drug with excipient, which may
41 subsequently affect the release from the carrier (Agrawal et al., 2009; McLennan et al., 1985; Menozzi et al., 1984).
42 One particular device that has shown success in the delivery of doxorubicin (Dox) is the DEB known as the DC
43 Bead™, which is used for the treatment of hypervascular tumours and arteriovenous malformations (Lewis et al.,
44 2007; Lewis et al., 2006; Taylor et al., 2007). The beads consist of a biocompatible polyvinyl alcohol (PVA)
45 hydrogel microsphere that has been modified with hydrophilic 2-acrylamido-2-methylpropane sulfonate sodium salt
46 moieties (AMPS), and is tinted blue to allow visualisation of drug loading (Lewis and Dreher, 2012; Lewis et al.,
47 2007; Lewis et al., 2006). The negatively charged sulfonate group enables the bead to complex with positively
48 charged drugs (Gonzalez et al., 2008). The beads are non-degradable and available in a range of sizes from 70-700
49 µm. These are supplied steam sterilised in a 10 mL colour-coded glass vial sealed with a rubber stopper and
50 aluminium cap. Each vial contains 2 mL of beads stored in 6 mL of phosphate buffered saline (PBS) and is intended
51 for single patient use. The size of the bead and the amount of the drug is selected according to the pathology of the
52 patient receiving treatment by a physician with appropriate experience in interventional oncology. The first size of
53 DC Bead™ to be evaluated in a clinical study was 500-700 µm and was based upon the predicate Gelfoam particles.
54 Gradually the size of DEBs has decreased, as smaller beads are favoured to achieve deeper tumour penetration and
55 increase the delivery of drug as a result of a larger bead surface area to volume ratio. This has led to the
56 development of 70-150 µm beads to meet the desire to deliver a large concentration of drug at a greater distal level
57 whereupon the drug is taken up by the bead through an ion-exchange mechanism (Gonzalez et al., 2008; Lewis et
58 al., 2007). When mixed with drug the beads change colour, which indicates the drug has been loaded. The beads
59 have a high water content of > 95 % which enables the diffusion of molecules into and out of the polymer structure.
60 Elution rate and uptake of the drug is dependent upon bead size, as diffusion is inversely proportional to bead size as
61 caused by the increase in surface area to volume ratio. The beads initially increase in diameter as they are
62 reconstituted in water, but then decrease as water is displaced from the bead and the drug is sequestered from the
63 solution (Taylor et al., 2007). The ion-exchange mechanism is a reversible reaction and upon contact with ions
64 contained in blood, the drug is released from the bead. DC Bead™ can be loaded with a recommended dose of 37.5
65 mg Dox/mL of hydrated bead, however generally a dose of 25 mg Dox/mL of beads is administered. Typically 2-4
66 mL is administered over a course of treatment, with a maximum of 150 mg of Dox per patient according to systemic
67 dose limits. Understanding the interaction of drug with the aforementioned polymeric carrier will allow optimisation
68 and modification of the design of a new type of drug delivery system for even more effective targeted drug therapy.
69 To achieve an understanding of the stoichiometry and thermodynamics of the drug-bead interactions requires the

70 application of a technique that can monitor binding phenomena in real-time and at specified temperatures.
71 Isothermal titration calorimetry has the potential to achieve this aim.

72 Isothermal titration calorimetry (ITC) is an established analytical technique used to investigate
73 thermodynamic information for a variety of chemicals and biological systems. ITC has previously been used to
74 investigate similar processes, such as polymer complexes (Zhang et al., 2015), drug-polymer interactions, surfactant
75 behaviour and drug-excipient ratios (Waters et al., 2010). More specifically, ITC has been employed to evaluate
76 intermolecular interactions with high sensitivity to establish the degree to which excipients become saturated with
77 drug, i.e. maximum drug loading capabilities (Waters et al., 2012; Waters et al., 2014). ITC is ideally suited to such
78 studies as it allows rapid and accurate determination of thermodynamic parameters such as binding constant (K),
79 reaction stoichiometry (N), enthalpy (ΔH), entropy (ΔS) and Gibbs free energy (ΔG) for any given system. Based on
80 previous research within the group it was anticipated that ITC could be applied to the study of drug-polymer
81 interactions to yield thermodynamic and stoichiometric data that cannot be measured using any other current
82 analytical technique. Thus, this specific drug-polymer combination was identified as a model system to exemplify
83 the applicability of the technique to such scenarios.

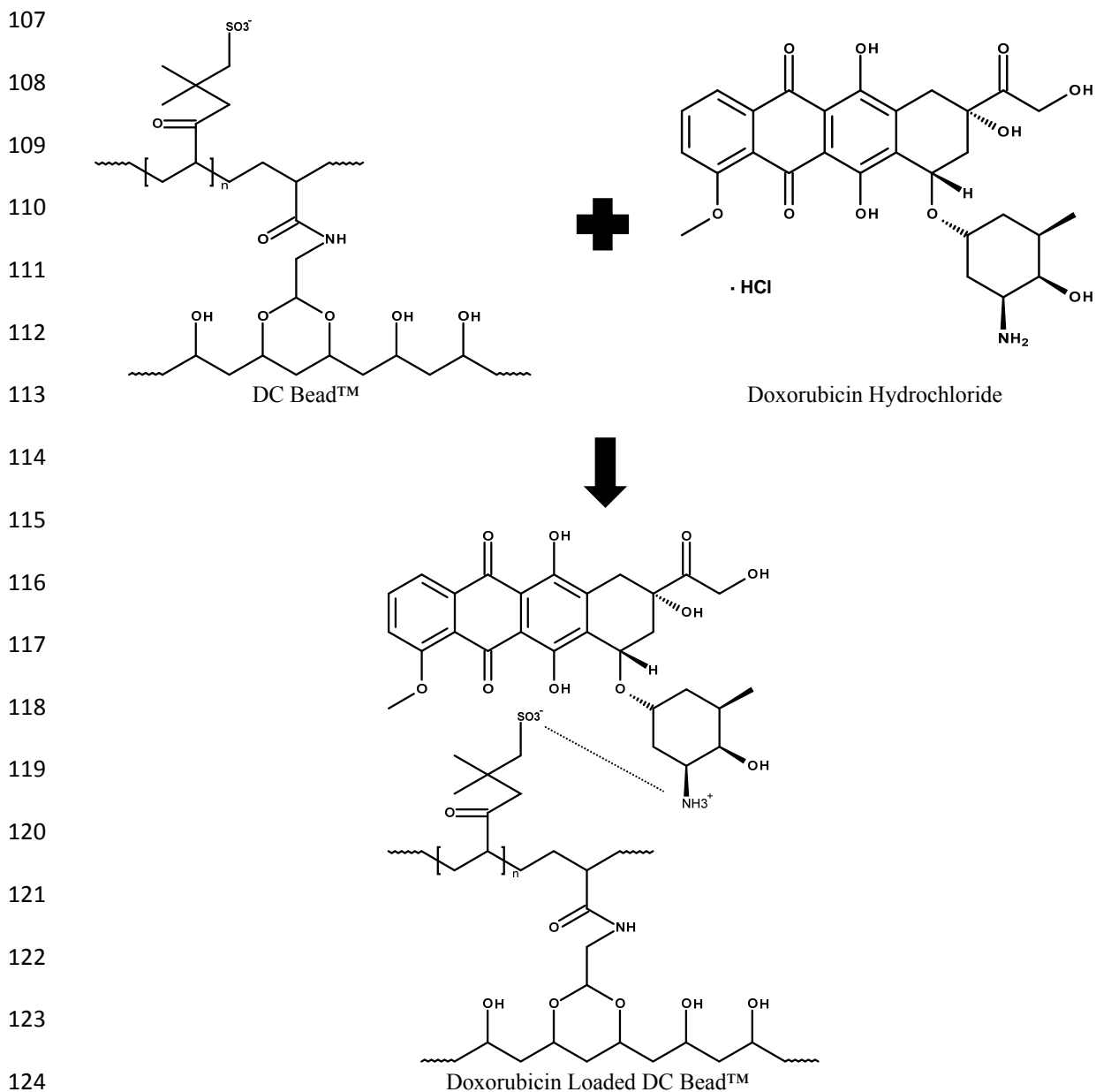
84 This paper describes the stoichiometric and calorimetric interactions between doxorubicin and the DC
85 Bead™ system at six specific temperatures to help identify the drug loading capacity of the systems under varying
86 conditions and the thermodynamics associated with the binding process.

87

88 **Materials and Methods**

89 Doxorubicin HCl (Hisun, China) and DC Bead™ of size 70 – 150 μm (DC BeadM1™) were kindly
90 donated by Biocompatibles UK Ltd, a BTG International group company (Camberley, UK), the latter supplied
91 sterile in sodium phosphate buffer as a 2 mL hydrated volume. This particular bead size was chosen for this study as
92 a model system as it was important to focus on one specific size range to minimise the variables encountered,
93 furthermore, it has the maximum surface area to volume ratio thus it was anticipated that it would undergo the most
94 rapid diffusion of drug through the bead. Buffer was removed and the beads were washed with deionised water then
95 centrifuged for 30 seconds at 3000 rpm prior to analysis. A Microcal isothermal titration calorimetric unit (ITC)
96 linked to a Microcal MCS observer was utilised for all experiments with Origin software for analysis. Initial dilution
97 experiments were conducted with the enthalpy of dilution for the addition of solvent to polymer subtracted from the
98 total enthalpies of reaction to remove dilution effects. The sample cell (total volume = 1.42 mL) comprised of an
99 aqueous solution containing 10 – 100 mg of beads with water (without buffer present) alongside a simple aqueous
100 solution in the reference cell. The 290 μL syringe contained 10 mM doxorubicin (58 mg/mL) and was stirred at 307
101 rpm, injecting a total of 14 injections at 20 μL per injection and 600 seconds between injections. These parameters
102 were chosen as they were found to give an appropriate signal. Experiments were conducted at six temperatures (293
103 to 313 K), all in triplicate to ensure reproducibility. The six temperatures were selected to cover typical room
104 temperatures encountered during sample preparation (i.e. prior to injection) through to a temperature just beyond

105 that encountered by the formulated product *in vivo*. Data were analysed to determine the stoichiometry of the drug-
106 polymer interaction (n) and enthalpy of binding (ΔH) associated with the interaction highlighted in Figure 1.



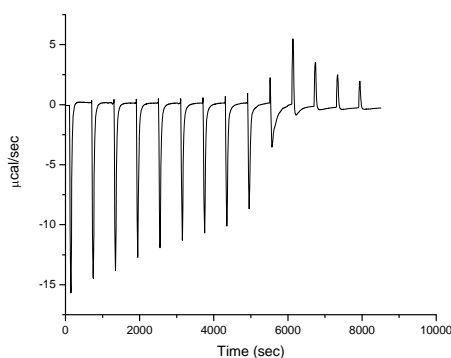
125 Figure 1: Structural formula of DC Bead™, Doxorubicin and the combined product.

126

127 Results and Discussion

128 After experimenting with several drug concentrations it was found that 10 mM doxorubicin produced the
129 most suitable ITC profile for injection, although a small demicellisation event was observed which then had to be

130 subtracted prior to further analysis. In contrast to this, the addition of aqueous solution to the sample cell containing
131 the beads appeared to be almost thermoneutral. Initially the enthalpic signal indicated the process of drug-polymer
132 binding to be an exothermic event, followed by endothermic demicellisation once the polymer had become saturated
133 with drug (Figure 2). The inflection point can clearly be seen after the first few injections indicating saturation of the
134 polymer had been achieved. In summary, the process observed in Figure 2 is assumed to be an initial drug-polymer
135 (exothermic) binding event for approximately the first ten injections, culminating in bead saturation, followed by
136 four drug demicellisation (endothermic) injections.



137

138 Figure 2: Raw ITC data for the injection of doxorubicin to aqueous solution at 298 K.

139

140 Similar profiles to Figure 2 were observed at all temperatures studied. Assuming each experiment
141 contained 100 mg of centrifuged beads (which are known to have a solid content of 3.03 %), which in turn is known
142 to contain 45.45 % 2-acrylamido-2-methylpropane sulfonate (AMPS), then it was possible to calculate the ratio of
143 drug to SO_3^- groups. It is assumed that the endpoint of exothermic signals was equivalent to when Dox saturated
144 AMPS binding sites. The volume of Dox titrated to achieve the exothermic endpoint was calculated by multiplying
145 the number of exothermic injections by the volume per injection (μL). The concentration of Dox ($\text{mg}/\mu\text{L}$) was then
146 multiplied by the volume of injections at the endpoint (μL), to equal the mass of Dox at the said endpoint (mg).
147 Since the mass of Dox had been calculated and the relative molecular mass is known ($(\text{C}_{27}\text{H}_{29}\text{NO}_{11}.\text{HCl})$ 579.99g),
148 the number of moles was calculated by dividing the mass of Dox bound to AMPS by the relative molecular mass of
149 Dox. The solid mass of beads in the sample cell was the weight of centrifuged beads used (mg) divided by 100 and
150 multiplied by the % solid content. However, Dox binds to AMPS groups' situated on the beads and not to the whole
151 bead structure. Each bead contains 45.45 % AMPS therefore, the mass of AMPS is equal to the mass of beads (mg)
152 divided by 100 and multiplied by 45.45 %. Since the mass of AMPS has been calculated and the relative molecular
153 mass is known ($(\text{C}_7\text{H}_{12}\text{NNaO}_4\text{S})$ 229.23g), the number of moles can be calculated by dividing the mass of AMPS by
154 the relative molecular mass of AMPS. Therefore moles of AMPS in the sample cell divided by the moles of Dox at

155 the endpoint equals the molar ratio of Dox binding to AMPS. A summary of the calculated data can be seen in Table
156 1 with an overall standard deviation of 0.02.

157 Table 1: A summary of calculated binding ratios from ITC data for the injection of doxorubicin into polymer beads
158 over a series of temperatures.

Temperature / K	Average no. Dox. per AMPS (± 0.02)
293	0.39
298	0.38
303	0.37
308	0.36
310	0.37
313	0.38

159
160 This is the first report of the investigation of the interaction of soluble drug and immobilised sulfonate on a
161 bead resin, yet similar reports have considered other drug-excipient interactions, for example, Yousefpour and co-
162 workers have used ITC to characterise the thermodynamic profile of doxorubicin-dextran interactions (Yousefpour
163 et al., 2011). Thermal analysis of Dox-dextran complexation revealed that each Dox molecule bound with 3 dextran
164 glycosyl monomers. In addition, Tian and co-workers characterised the binding of Dox with various types of
165 pluronic-based co-polymer systems using ITC. Results revealed the strong electrostatic interactions between the –
166 COOH group of pluronic-PAA with the positively charged doxorubicin at physiological pH (Tian et al., 2007a). ITC
167 showed clearly the pH effect on the Dox-polymer binding. The electrostatic interactions were found to be the
168 predominant factor for the Dox/pluronic-PAA complex formation, while the shielding effect of NaCl on the
169 positively charged amino group and negatively charged COOH decreased the strength of interactions (Tian et al.,
170 2007b). It is thought that a similar interaction is being observed in this study, i.e. the drug is forming a complex with
171 the SO_3^- groups within the polymer. The lack of significant variation in ratio with temperature implies there is no
172 temperature-induced conformational change in the polymer in solution resulting in a change in the number of
173 ‘available’ SO_3^- groups. This may not be overly surprising, as the bead matrix is cross-linked and polymer chain
174 rearrangement will be restricted.

175 Through analysis of the area under the curves for the data presented (and their replicates) up to the points of
176 inflection permitted determination of the change in enthalpy associated with the binding event to be determined, as
177 summarised in Table 2.

178

179

180

181

182 Table 2: A summary of calculated changes in enthalpy upon binding from ITC data for the injection of doxorubicin
183 into polymer beads over a series of temperatures.

Temperature / K	Average change in enthalpy upon dox.- bead binding (kJ/mol)
293	- 14.8 (± 2.4)
298	- 15.9 (± 2.5)
303	- 15.8 (± 0.4)
308	- 17.8 (± 0.7)
310	- 21.4 (± 3.1)
313	- 19.4 (± 1.3)

184

185 For binding at 293 K the mean enthalpy change was found to be $- 14.8 \text{ kJmol}^{-1}$ whereas at 313 K was $-$
186 19.4 kJmol^{-1} . Although small differences were observed between the temperatures, overall the binding phenomenon
187 was not dramatically affected by an increase in experimental temperature of 20 K. When these values are compared
188 with data for interactions of a similar nature, such as diminazene aceturate with poly(aspartic acid), it can be
189 confirmed that the values obtained in this work correspond well (Govender et al., 2000), whereby the authors state
190 the interaction was identified to be enthalpically-driven through the formation of hydrogen bonds. Furthermore, the
191 same thermodynamic conclusion can be drawn, in that the high enthalpy change of reaction compensates for a small
192 entropic change with the additional observation that the process is only affected by temperature over the range
193 studied to a small extent. Although in some cases it would appear there are small differences between the values,
194 once the error limits are included the actual change in enthalpy can be considered to be almost constant. Any minor
195 differences that have been recorded are most likely a result of small rearrangements of surrounding solvent
196 molecules rather than as a direct result of variations in drug-bead interactions.

197 However, the findings from this study were not as straightforward as initially expected when considering
198 the drug to polymer ratio values obtained. Data obtained from previous work on the same drug-bead system (using
199 UV analysis) has demonstrated binding of 37.5 mg per mL of bead, which equates to a Dox:AMPS ratio of 0.7:1
200 (and is unanimously accepted as an achievable value in clinical practice equating to 150 mg in 4 mL of beads, i.e.
201 the standard recommended dose per administration for patients per treatment). Indeed, ratios of 1:1 are also
202 achievable with higher doxorubicin loading concentrations (Fajardo, 2006), whereas in this study, the ratio was
203 closer to 0.4:1. The difference between these two ratios is most likely a result of differing methods of mixing, the
204 clinical approach routinely involves the complete, rapid addition of drug to bead whereas the ITC method is small,
205 sequential additions. This alternative, latter approach may induce conformational changes within the beads that
206 prevent further drug accessing the binding sites, keeping the ratio lower than that observed using UV analysis.

207

208 **Conclusions**

209 In summary, this study has determined the suitability of ITC to investigate drug-excipient interactions such
210 as those between drugs and polymer beads. Moreover, it has been possible to calculate the ratio of drug molecules to
211 SO_3^- groups which appears to be temperature independent and the change in enthalpy with binding is also unaffected
212 by temperature effects. The reasons for differences between the results presented here and UV data are the subject of
213 current research. Finally, it would be of interest to investigate the same bead system with a series of compounds to
214 determine the specificity of the binding process, such as that observed by others for related polymer-drug systems
215 (Govender et al., 2000). To maximise the efficiency of drug delivery systems, such as those studied in this work, it is
216 essential to fully understand their physicochemical behaviour and then, based on this knowledge, it may be possible
217 to enhance the amount that can be contained within the polymer by maximising drug-polymer interactions. Although
218 the experimental protocol was somewhat dissimilar to that seen in clinical applications, i.e. small aliquots were
219 added over many hours rather than the standard mixing time of 30 minutes prior to injection, it still yields useful
220 information with respect to drug-bead interactions.

221

222 **Acknowledgements**

223 The authors wish to thank Biocompatibles UK Ltd for funding this study.

224

225 **References**

- 226 Agrawal, P., Barthwal, S.K., Barthwal, R., 2009. Studies on self-aggregation of anthracycline drugs by
227 restrained molecular dynamics approach using nuclear magnetic resonance spectroscopy supported by
228 absorption, fluorescence, diffusion ordered spectroscopy and mass spectrometry. *European journal of*
229 *medicinal chemistry* 44, 1437-1451.
- 230 Fajardo, M.V.G., 2006. *Delivery of Drugs from Embolisation Microspheres*, Univeristy of Brighton.
- 231 Gonzalez, M.V., Tang, Y., Phillips, G.J., Lloyd, A.W., Hall, B., Stratford, P.W., Lewis, A.L., 2008. Doxorubicin
232 eluting beads - 2: Methods for evaluating drug elution and in-vitro:in-vivo correlation. *Journal of*
233 *Materials Science: Materials in Medicine* 19, 767-775.
- 234 Govender, T., Riley, T., Ehtezazi, T., Garnett, M.C., Stolnik, S., Illum, L., Davis, S.S., 2000. Defining the
235 drug incorporation properties of PLA-PEG nanoparticles. *International Journal of Pharmaceutics* 199, 95-
236 110.
- 237 Kerr, D.J., 1987. Microparticulate drug delivery systems as an adjunct to cancer treatment. *Cancer Drug*
238 *Delivery* 4, 55-61.
- 239 Lewis, A.L., Dreher, M.R., 2012. Locoregional drug delivery using image-guided intra-arterial drug eluting
240 bead therapy. *Journal of Controlled Release* 161, 338-350.

241 Lewis, A.L., Gonzalez, M.V., Leppard, S.W., Brown, J.E., Stratford, P.W., Phillips, G.J., Lloyd, A.W., 2007.
242 Doxorubicin eluting beads - 1: Effects of drug loading on bead characteristics and drug distribution.
243 *Journal of Materials Science: Materials in Medicine* 18, 1691-1699.

244 Lewis, A.L., Gonzalez, M.V., Lloyd, A.W., Hall, B., Tang, Y., Willis, S.L., Leppard, S.W., Wolfenden, L.C.,
245 Palmer, R.R., Stratford, P.W., 2006. DC Bead: In vitro characterization of a drug-delivery device for
246 transarterial chemoembolization. *Journal of Vascular and Interventional Radiology* 17, 335-342.

247 Liu, Z., Cheung, R., Wu, X.Y., Ballinger, J.R., Bendayan, R., Rauth, A.M., 2001. A study of doxorubicin
248 loading onto and release from sulfopropyl dextran ion-exchange microspheres. *Journal of controlled*
249 *Release* 77, 213-224.

250 McLennan, I., Lenkinski, R., Yanuka, Y., 1985. A nuclear magnetic resonance study of the self-association
251 of adriamycin and daunomycin in aqueous solution. *Canadian journal of chemistry* 63, 1233-1238.

252 Menozzi, M., Valentini, L., Vannini, E., Arcamone, F., 1984. Self-association of doxorubicin and related
253 compounds in aqueous solution. *Journal of pharmaceutical sciences* 73, 766-770.

254 Natarajan, J.V., Nugraha, C., Ng, X.W., Venkatraman, S., 2014. Sustained-release from nanocarriers: A
255 review. *Journal of Controlled Release* 193, 122-138.

256 Rana, D.K., Dhar, S., Sarkar, A., Bhattacharya, S.C., 2011. Dual Intramolecular Hydrogen Bond as a Switch
257 for Inducing Ground and Excited State Intramolecular Double Proton Transfer in Doxorubicin: An
258 Excitation Wavelength Dependence Study. *The Journal of Physical Chemistry A* 115, 9169-9179.

259 Shuhendler, A.J., Cheung, R.Y., Manias, J., Connor, A., Rauth, A.M., Wu, X.Y., 2010. A novel doxorubicin-
260 mitomycin C co-encapsulated nanoparticle formulation exhibits anti-cancer synergy in multidrug
261 resistant human breast cancer cells. *Breast cancer research and treatment* 119, 255-269.

262 Taylor, R.R., Tang, Y., Gonzalez, M.V., Stratford, P.W., Lewis, A.L., 2007. Irinotecan drug eluting beads for
263 use in chemoembolization: In vitro and in vivo evaluation of drug release properties. *European Journal*
264 *of Pharmaceutical Sciences* 30, 7-14.

265 Tian, Y., Bromberg, L., Lin, S., Alan Hatton, T., Tam, K.C., 2007a. Complexation and release of doxorubicin
266 from its complexes with pluronic P85-b-poly (acrylic acid) block copolymers. *Journal of controlled*
267 *Release* 121, 137-145.

268 Tian, Y., Ravi, P., Bromberg, L., Hatton, T.A., Tam, K.C., 2007b. Synthesis and aggregation behavior of
269 Pluronic F87/poly (acrylic acid) block copolymer in the presence of doxorubicin. *Langmuir* 23, 2638-
270 2646.

271 Waters, L.J., Bedford, S., Parkes, G., Mitchell, J., 2010. Influence of lipophilicity on drug-cyclodextrin
272 interactions: A calorimetric study. *Thermochimica Acta* 511, 102-106.

273 Waters, L.J., Hussain, T., Parkes, G., 2012. Titration calorimetry of surfactant-drug interactions: Micelle
274 formation and saturation studies. *The Journal of Chemical Thermodynamics* 53, 36-41.

275 Waters, L.J., Hussain, T., Parkes, G.M.B., 2014. Thermodynamics of micellisation: Sodium dodecyl
276 sulfate/sodium deoxycholate with polyethylene glycol and model drugs. *The Journal of Chemical*
277 *Thermodynamics* 77, 77-81.

278 Yousefpour, P., Atyabi, F., Farahani, E.V., Sakhtianchi, R., Dinarvand, R., 2011. Polyanionic carbohydrate
279 doxorubicin-dextran nanocomplex as a delivery system for anticancer drugs: in vitro analysis and
280 evaluations. *International journal of nanomedicine* 6, 1487-1496.

281 Zhang, H., Zeeb, B., Salminen, H., Weiss, J., 2015. Isothermal titration calorimetric analysis on
282 solubilization of an octane oil-in-water emulsion in surfactant micelles and surfactant-anionic polymer
283 complexes. *Journal of Colloid and Interface Science* 438, 7-13.

284

285