pH-Responsive Liposomes Self-Assembled from Electrosprayed Microparticles, and Their Drug Release Properties

Kailin Liu [1], Heyu Li [1], Gareth R. Williams [2,*], Junzi Wu [1], Li-Min Zhu [1,3,*]

1. College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai, 201620, China

2. UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London, WC1N 1AX, UK

3. Key Lab of Science & Technology of Eco-Textile, Ministry of Education, Donghua University, China

* Correspondence authors:

E-mail: lzhu@dhu.edu.cn (L.-M. Zhu); g.williams@ucl.ac.uk (G. R. Williams).

Tel：+86 216 779 2655 (L.-M. Zhu); +44 (0) 207 753 5868 (G.R. Williams)

Abstract: In this work, composite Eudragit L100 / phosphatidyl choline microparticles were fabricated through electrospraying. pH-responsive liposomes were found to self-assemble from these when the microparticles were added into aqueous media. The microparticles and the liposomes were both approximately spherical in shape according to electron microscopy, but the liposomes have much smaller diameters (200-300 nm) than the electrosprayed particles (1.6-1.7 μm). The zeta potential of the liposomes is approximately -30 mV, which suggests the formation of stable suspensions. Varying the pH conditions used for self-assembly causes the liposomes to change their shape and structure, due to the influence of Eudragit molecules. The model drug ketoprofen could be loaded into the liposomes, with an entrapment efficiency of 75%. pH-dependent release was observed from the drug-loaded liposomes. At pH 4.5, only 58% of the drug loaded was released after 12 hours while 80% was released at pH 7.4. Overall, these results demonstrate that the pH-dependent liposomes developed have great potential for application as stimuli-responsive drug delivery systems.

Keywords: pH-dependent liposome; electrosprayed microparticle; self-assembly; oral drug delivery

1. Introduction

In recent years, a range of pH-sensitive liposomes, which can control the release of a loaded drug in response to the pH of the surrounding medium, have been reported [1-3]. These liposomes can serve as carriers of many different active ingredients, for instance small organics, peptides, RNA, DNA, and diagnostic agents [4-7]. They also generally have good biocompatibility [8]. There are two key types of pH-dependent liposomes, which are bounded either by natural lipid bilayers or polymer functionalized lipid bilayers. A pH-driven phase transition of the lipid bilayers or of the polymer attached to them permits their contents to be freed into solution in a responsive manner [9]. However, natural phospholipid-based liposomes usually have low stability at low pH values [10]. It is therefore desirable to prepare polymer-functionalized systems to improve stability.

Many studies have reported that liposomes decorated with pH-sensitive polymers are capable of pH-responsive release [11-13]. Eudragit L100, which is synthesized from methacrylic acid and methacrylic acid methyl ester, is a pH-sensitive polymer widely used in the pharmaceutical industry, for instance as enteric coatings for tablets. It can also be employed for preparing microspheres and nanoparticles for use as targeted gastro-intestinal drug delivery systems [14, 15]. Eudragit L100 dissolves only at pH values higher than 6.0, and is insoluble in aqueous media below this pH. Thus, it can be specifically applied to release an incorporated drug only in the lower parts of the gastro-intestinal tract [16].

A number of methods can be applied to obtain pH-sensitive liposomes. For instance, Straubinger et al. used oleic acid and phosphatidylethanolamine to fabricate pH-sensitive liposomes via the evaporation method [17]. In other work, Catalan-Latorre et al. fabricated multicompartment liposomes loaded with curcumin through combining Eudragit S100, hyaluronic acid and a phospholipid using the freeze-drying method [18]. However, these methods can be complex, and often there is solvent residue in the multicomponent liposomes. As a result, researchers have sought alternative methods for producing liposomes [19-21].

Liposomes form as a result of molecular self-assembly, driven by noncovalent interactions. The spontaneous association of amphiphilic molecules, isolating lipophilic sections from an aqueous medium, for instance, leads to the creation of stable and well-defined supramolecular structures [22].

This approach is important for the fabrication of biomaterials [23-25], but it is challenging to control such a bottom-up process. Therefore, methods to direct the contacts between building blocks and drive the assembly process towards a desired conclusion are required.[26-28].

One route that may be used to obtain control over self-assembly processes is electrospraying, a hydrodynamic atomization approach. This is a top-down process which exploits electrical energy to evaporate the solvent from a polymer solution, resulting in solid dispersions in the form of sub-micron-sized particles. Electrospinning is a similar technique, and yields nanoscale fibers. Both electrosprayed particles and electrospun fibers can be exploited as templates to direct the self-assembly of nanoscale-objects from multiple components [29, 30]. Self-assembly is achieved by a simple dissolution of the fiber or particle precursors, and the resulting aggregates can easily be loaded with an active ingredient during the assembly process. For instance, Yu et al. prepared core/shell nanofibers by electrospinning, and were able to use these to self-assemble drug-loaded nanoparticles with controllable sizes [31]. Jin and co-workers have also prepared thermosensitive ketoprofen-loaded liposomes via the dissolution of electrosprayed composite microparticles of poly(N-isopropylacrylamide) and phosphatidyl choline [19]. During dissolution, the polymer matrix (which is typically made of a hydrophilic fast dissolving polymer) is believed to help confine the assembling components in close proximity, facilitating their self-aggregation to minimize any interactions between the aqueous medium used for assembly and hydrophobic components in the composites. The liposomes fabricated in this way are generally found to have uniform diameters, and high drug entrapment efficiencies. The fact that they are produced on demand from stable solid dispersions means that the stability issues commonly arising with liposomal formulations can be effectively ameliorated.

The studies reported to date on stimuli-responsive liposomes have generally employed the evaporation method, and there is little work on using electrospinning or electrospraying to this end [19, 32]. In this study, we sought to exploit electrospraying to generate microparticles of Eudragit L 100 loaded with phosphatidyl choline (PC). When the microparticles were added to water, the PC was found to self-assemble into liposomes, and the physicochemical and biological properties of the liposomes were studied in detail. Ketoprofen-loaded systems were further prepared, and their drug release properties

examined.

2. Experimental

2.1 Materials

Eudragit L100 (average molecular weight ca. 135,000) was provided by Rohm GmbH (Darmstadt, Germany). Phosphatidyl choline (PC, extracted from soybean) was procured from the Sinopharm Chemical Reagent Co. (Shanghai, China). Chloroform and N,N-dimethylacetamide were purchased from the Traditional Industries Co. (Shanghai, China). Ketoprofen (KET) was obtained from Shanghai Greentech Industries Co. (Shanghai, China). Water was double distilled prior to use.

2.2 Electrospraying

Solutions were prepared by adding Eudragit L100 and PC to a mixture of chloroform/N,N-Dimethylacetamide (4:1 v/v) at room temperature and stirring for over 20 h. Details of the solution compositions are given in Table 1. The fully dissolved solutions were then loaded in 5 mL syringes, which were fitted with a stainless-steel flat-tipped needle (internal diameter 0.5 mm). The syringes were mounted on a syringe pump (KDS100, Cole-Parmer, Vernon Hills, IL, USA) and solution expelled at a rate of 1.0 mL/h. A voltage of 16 kV (ZGF- 2000 power supply, Shanghai Sute Electrical Co. Ltd., China) was supplied between the spinneret and a flat aluminium foil-covered collector (10×10 cm). The tip-to-collector distance was set at 25 cm, and experiments performed at 25 ± 2 ºC and relative humidity of 50 ± 5%. Six formulations were prepared in total (see Table 1).

Table 1 The compositions of the solutions used for electrospraying

Concentration	F_1	F_2	F_3	F_4	F_5	F_6
$C_{Eudragit}$ (%w/v)	2	2	2	2	2	2
C_{PC} (%w/v)	0	0.5	1	1.5	2	1
C_{KET} (%w/v)	0	0	0	0	0	0.4

$C_{Eudragit}$ denotes the concentration of Eudragit, C_{PC} the concentration of PC, and C_{KET} the concentration of KET.

2.3 Preparation of liposomes

0.01 g of the electrosprayed particles were removed from the collector and added to 100 mL of phosphate buffered saline (PBS, pH=7.4) at room temperature.

2.4 Characterization of microparticles

The surface morphology of the microparticles was observed using a JSM-5600LV scanning electron microscope (SEM; JEOL, Tokyo, Japan). The average particle diameter was calculated through measuring more than 100 different particles in SEM images using the ImageJ software (National Institutes of Health, Bethesda, MA, USA).

The physical form of the components in the microparticles and their interactions were analyzed by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). FTIR was performed using a Nicolet-Nexus 670 FTIR spectrometer (Nicolet Instrument Corporation, Madison, WI, USA). XRD was undertaken on a D/Max-BR diffractometer (Rigaku, Tokyo, Japan) with Cu-Kα radiation. Thermogravimetric analysis (TGA; STA409PC instrument, Netzsch, Selb, Germany) was also performed to determine the thermal stability of the microparticles. 3-5 mg of dried sample was weighed into a crucible and analysis was carried out at a heating rate of 10 ºC/min under a flow of nitrogen.

2.5 Characterization of liposomes

The morphology of the liposomes was evaluated by transmission electron microscopy (TEM; H-800 instrument, Hitachi, Tokyo, Japan). One drop of the liposome suspension (microparticles dissolved in phosphate buffered saline, pH=7.4) was dropped onto a carbon-coated copper grid and dried at 25 ºC. Particle size and zeta potential were measured with a Zetasizer Nano ZS instrument (Malvern Instruments, Malvern, UK). 0.02 g of the microparticles were added to 100 mL of PBS. As a control, pure PC was added to PBS at the same concentration (0.02 g in 100 mL) and the zeta potential and size recorded. In order to study the behavior of the liposomes under different pH conditions, the pH of the liposome suspensions was also adjusted to 4.5, 5.5, 6.0 and 6.5

2.6 Drug entrapment efficiency

The encapsulation efficiency (EE) of KET in the self-assembled liposomes from F6 was calculated as follows:

$EE = (W_t - W_f)/ W_t \times 100\%$

where W_t is the total drug mass in the electrosprayed particles and W_f the amount of free drug in the supernatant after liposome formation. The latter was isolated by ultracentrifugation (TGL-16G instrument, Anting Instrument Co. Ltd, Shanghai, China) for 30 min at 8,000 rpm, and the free drug content quantified by UV-vis spectrometry (UV-2101 spectrometer, Unico Instrument Co. Ltd, Shanghai, China) at 260 nm.

2.7 *In vitro* drug dissolution tests

For *in vitro* drug release experiments, 100 mg of the F6 microparticles were added to 10 mL of PBS buffer (pH 7.4) to form liposomes, and 3 mL of the liposome suspension then placed in a dialysis bag (MWCO=3500 Da). The filled dialysis bag was in turn immersed in a plastic bottle filled with 25 mL of PBS (pH = 7.4) or acetic acid buffer (pH = 4.5). The bottle was incubated at 37 ºC in a shaker bath operating at 100 rpm. Periodically, 1 mL aliquots were removed and replaced with the same volume of prewarmed buffer solution. Experiments were carried out in triplicate and the results are shown as mean ± S.D.

3. Results and discussion

3.1 Morphology of electrosprayed microparticles

The ease by which polymer solutions can be processed by electrohydrodynamic approaches is dependent on their concentrations and molecular weights, which ultimately determine the viscosity and conductivity [33]. The molecular weight of PC is low (< 1000 Da), and thus it cannot be independently processed by electrospraying. SEM images of the product obtained from an attempt to process a 4 % w/v solution of PC in a mixture of chloroform and N,N-Dimethylacetamide (4:1 v/v) are shown in the Supplementary Information, Fig S1. A few irregularly shaped droplets can be seen, but there are no microparticles present. The addition of Eudragit L100 to the solution increases the solution viscosity and ensures that sufficient chain entanglements can occur to generate microparticles with well-defined morphologies. The presence of PC can however improve the morphology of the microparticles (over a pure Eudragit solution), since it acts as a surfactant [34].

SEM images and size distribution of the electrosprayed microparticles (F1-F6) are given in Fig 1, with the details of their composition listed in Table 1. Most of the particles are spherical, with the exception of F1 where some irregularities are observed. In all cases there are a few fibers present as well as the particles. PC is an amphoteric ionic surface active agent. The addition of PC can lower the viscosity and surface tension of the electrospraying solution, which is conducive to the formation of spherical microparticles. The surfactant properties of PC are thought to contribute to the greater regularity of F2 as compared to F1 [35], as a result of its effect on the surface tension of the spinning solution. The average diameter of all the PC containing particles is around 1.6 μm, with F1 being a little smaller at 1.2 μm. A comparison of F3 and F6, which are identical except for the presence of drug in the latter, reveals that the two materials are virtually indistinguishable, and thus the presence or absence of KET has little influence on the particle size. All the microparticles have approximately the same spherical shape, indicating that no significant phase separation occurred in the process.

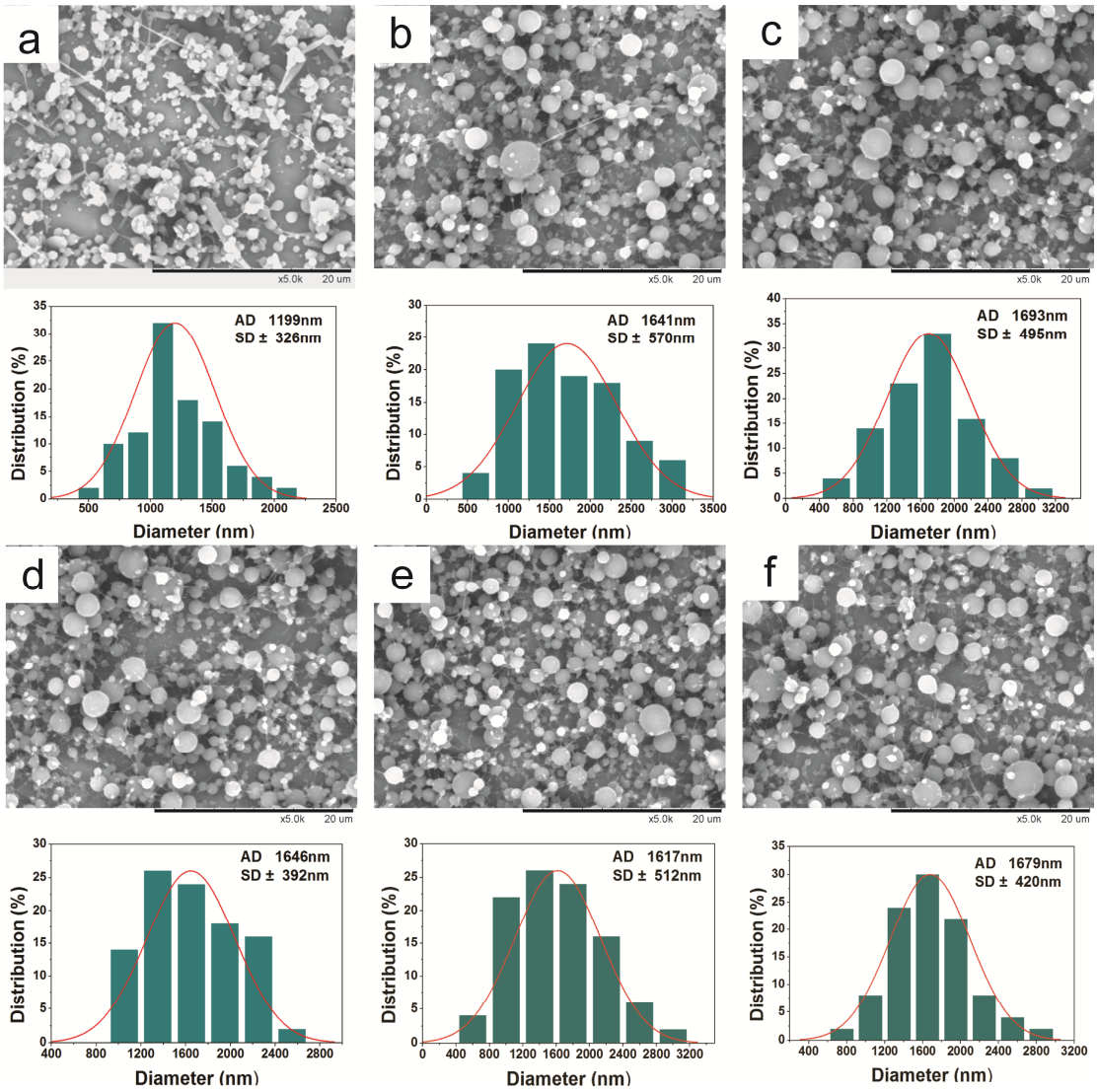

Figure 1 SEM images and diameter distributions of the electrosprayed particles. (a) F1, (b) F2, (c) F3, (d) F4, (e) F5, and (f) F6.

3.2 Physical form of the components in the microparticles

FTIR spectra are presented in Fig 2 (a). The particles of pure Eudragit (F1) show peaks at 1260 cm^{-1} and 1178 cm^{-1}, which corresponded to the ester (C-O-C) stretching bands. A strong absorption at 1728 cm^{-1} is caused by the stretching of the carbonyl groups. In the spectrum of PC, vibrations at 2924 and 2853 cm^{-1} correspond to symmetric and antisymmetric CH_2 stretching from the hydrophobic tail regions. Peaks at 1250 and 1047 cm^{-1} are the result of phosphate group stretching vibrations, and the band at *ca.* 1739 cm^{-1} arises from the carbonyl group in PC.

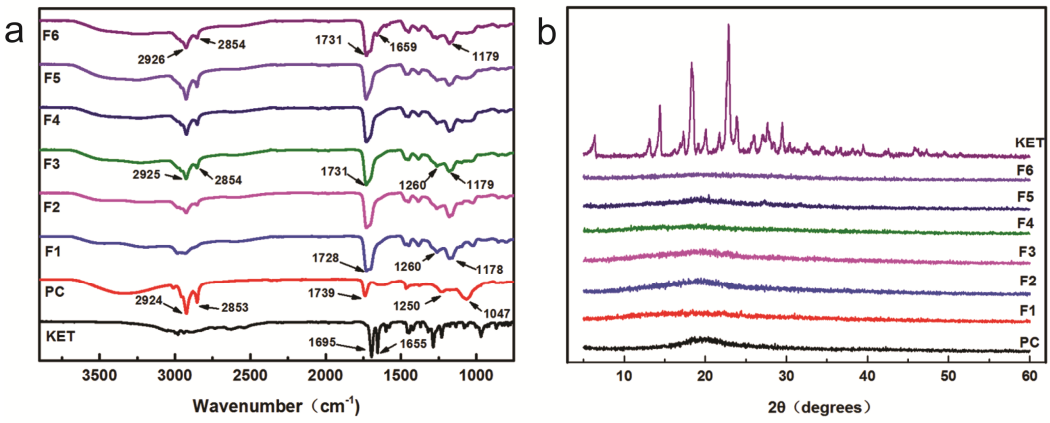

Figure 2 FTIR spectra (a) and X-ray diffraction patterns (b) of the electrosprayed particles.

The spectra of the Eudragit/PC electrosprayed particles (F2 – F5) contain all the characteristic peaks of both PC and Eudragit. However, some small shifts in frequency (for instance of the C=O group at around 1730 cm^{-1}) arise, suggesting interactions between two components. There are much larger differences between the spectra of KET and the KET/Eudragit/PC particles (M6). Absorption bands of KET at 1655 and 1695 cm^{-1} can be attributed to the C=O group, with the latter corresponding to the dimer present in the crystalline form of the drug. After processing into microparticles, peaks at 1731 cm^{-1} and 1659 cm^{-1} are visible. The small shifts in the positions of the Eudragit and KET vibrations suggest the presence of intermolecular interactions between them. In addition, the absence of the 1695 cm^{-1} band indicates that the KET dimer is not present in the particles, suggesting that KET-KET interactions are replaced by interactions with the Eudragit or PC.

The physical form of the components in the particles was also investigated by X-ray diffraction. The results are shown in Fig 2 (b). KET has a large number of distinct Bragg reflections visible in its pattern, demonstrating that it is a crystalline material. However, the Eudragit and PC materials are clearly amorphous, with only broad humps present. The patterns of all the PC loaded microparticles (F2 – F5) also show merely the characteristic humps of amorphous systems. In the KET-loaded particles (F6), no Bragg reflections corresponding to the crystalline drug can be seen, which indicates that the electrospraying process has converted KET into the amorphous form. This finding is in complete agreement with numerous other studies in the literature [36, 37].

Fig 3 illustrates the thermogravimetric (TG) and derivative thermogravimetric (DTG) curves for PC,

F1, F3 and F6 (data for the remaining systems are shown in Fig. S2). There are three main stages in the profiles for all the samples: below 200 ºC, between 200-400 ºC and above 400 ºC. The first is due to the loss of physisorbed water. The second, most significant, loss can be attributed to the combustion of the organic species present in the sample. When the temperature is between 400-900 ºC, the mass of the samples remains essentially constant. The temperature of maximum decomposition rate of PC is at 331 ºC, while those of Eudragit (F1) and PC-containing (F3) particles are at 376 and at 396 ºC, respectively. The addition of PC clearly has some effect on the thermal stability of the particles, although this is unlikely to be significant in the pharmaceutical setting given the elevated temperatures needed to drive decomposition. All the PC-containing particles exhibit the same behavior (see Fig 3 and Fig S2), and it can be seen that neither the amount of PC incorporated nor the presence of KET has any appreciable effect on the thermal stability.

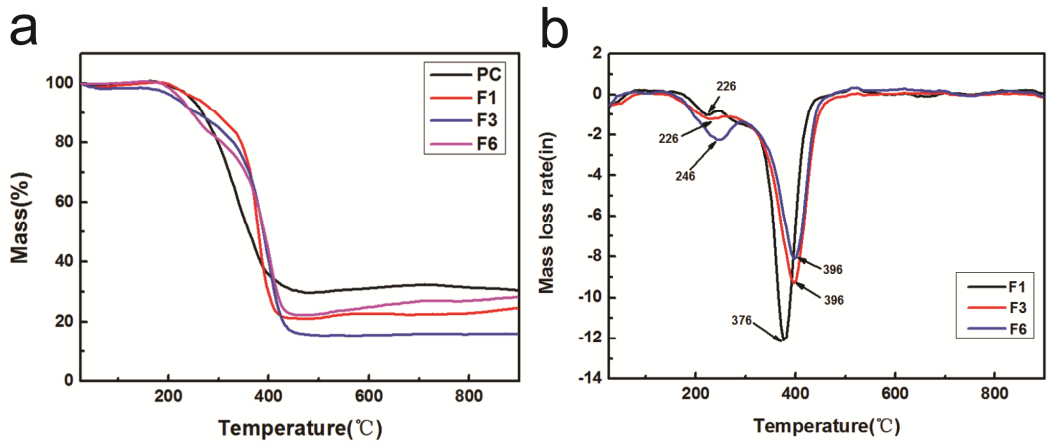

Figure 3 Thermal analysis of selected microparticles: (a) TG and (b) DTG.

3.3 Liposome formation

The formation of self-assembled liposomes from electrosprayed microparticles has been reported previously[19]. In agreement with previous research, when 0.1 g of the electrosprayed microparticles was added into 100 mL of PBS at pH 7.4, we observed the self-assembly of liposomes. The morphology and size of the liposomes were assessed by TEM and dynamic light scattering (DLS). The results are given in Fig 4 and Fig 5(a). In the TEM images (Fig 4), roughly spherical objects with core-shell structures can be seen. This indicates that there is some Eudragit polymer attached to the surface of the liposomes. The liposomes appear to be well dispersed, with sizes around 200 nm.

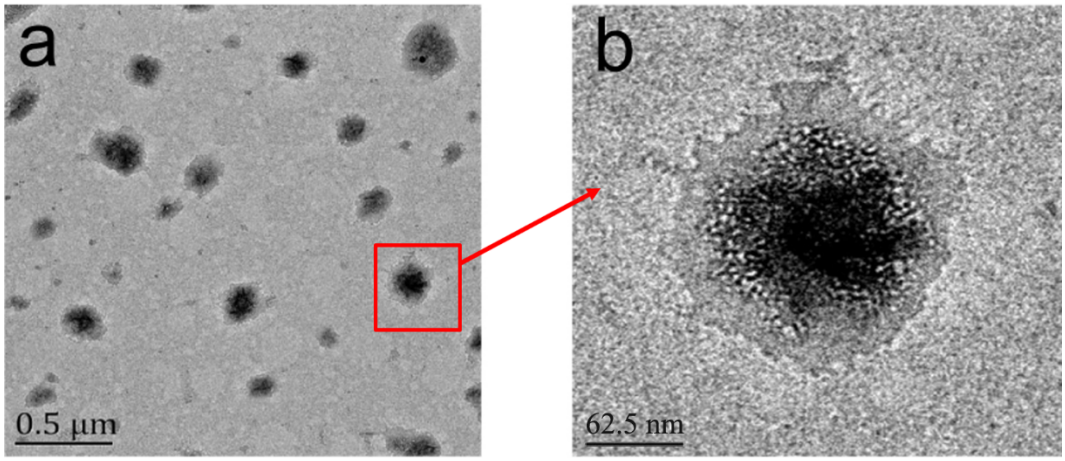

Figure 4 (a) a TEM image of liposomes assembled from F3 with (b) an enlarged image of single liposome.

The results obtained from DLS agree well with the TEM data. The data depicted in Fig. 5(a) for PC, F3 and F6 show that the liposomes self-assembled from the Eudragit-based particles are rather larger than those made of PC alone. This is consistent with the suggestion from TEM that the liposomes from F3 and F6 are decorated with Eudragit at their surfaces. The average diameter of the F3 liposomes from DLS was *ca.* 224 nm, which is slightly larger than suggested from TEM. We believe that is caused by the increased degree of hydration in DLS. A comparison of the liposomes assembled from S3 and S6 reveals that the addition of KET has little influence on the liposome diameters.

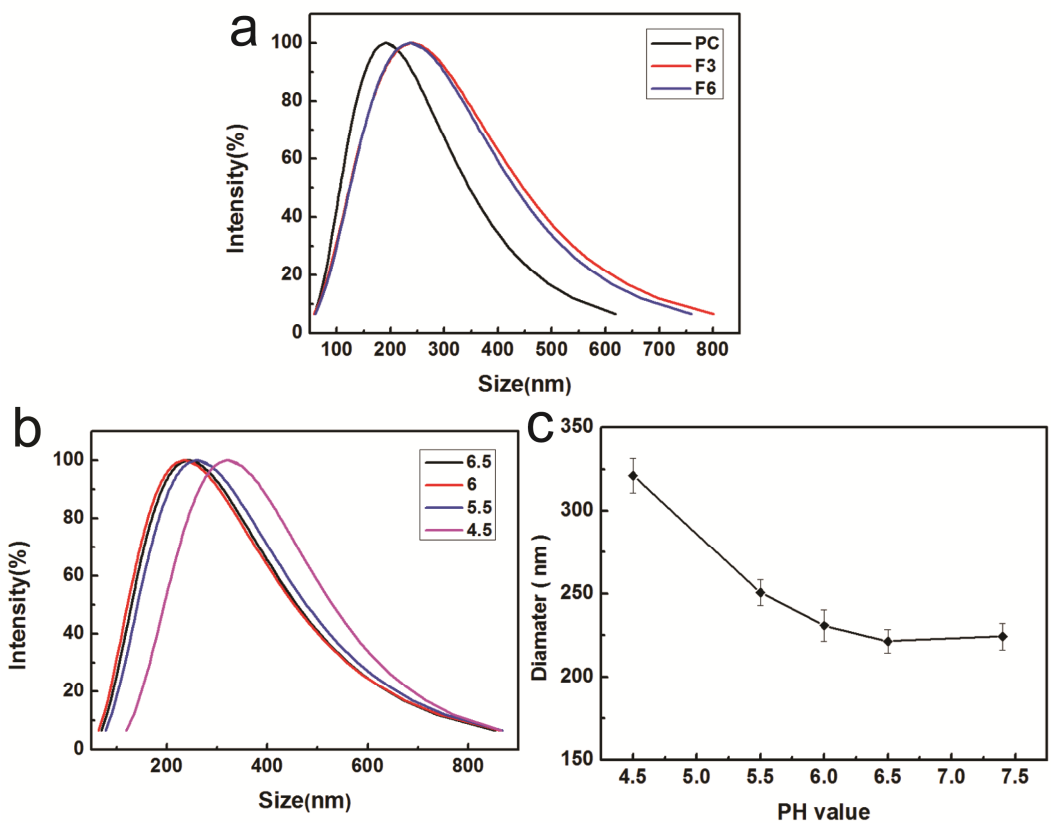

Figure 5 DLS measurements recorded on the self-assembled liposomes: (a) size measurements from pure PC, F3 and F6 in PBS (pH=7.4), (b) size measurements on liposomes from F3 when the buffer pH was adjusted to different values, and (c) the mean diameters of liposomes obtained from F3 at different pH.

The effect of a change in buffer pH on the liposome diameters was also measured, and the results are given in Fig 5(b) and (c). The mean diameters of the liposomes rise as the pH is decreased from 7.4 to 4.5 (Fig 5(c)). This might tentatively be ascribed to changes in the ionization of the COOH groups of Eudragit and their interactions with water molecules [38, 39]. A lower pH value will result in less ionization, and thus reduced solubility of Eudragit L 100 and larger mean diameters of the liposomes as the Eudragit chains aggregate at the liposome surfaces. Zeta potential values were recorded at pH 7.4 (Table S1). It should be noted that the Eudragit-only particles and PC both gave negative values, and the liposomes from F3 – F6 possessed even more negative zeta potentials (at around -30 mV), indicating that the liposome suspensions should have good colloidal stability. Zeta potential values were also recorded at pH 4.5 (Table S2), and it was found that although the zeta potentials have increased they are still around – 20 mV at this lower pH. Thus, the liposomal suspension will be stable

at both pH 4.5 and 7.4.

3.4 *In vitro* drug release

The entrapment efficiency of KET into the liposomes assembled from F6 in deionized water was determined to be approximately 75.0 ± 5.26%, which is higher than that of liposomes prepared using other methods[40]. This is in agreement with other reports that using electrospinning or spraying to prepare sacrificial templates for liposomal self-assembly can lead to improved drug entrapment efficiency [41, 42].

Drug release profiles obtained at pH 7.4 and at pH 4.5 are shown in Fig 6. In the drug release experiment, F6 particles were dissolved in PBS, forming liposomes containing KET. They were then added to two different pH buffer solutions at 37 °C. At pH=7.4, KET release reached a plateau of around 80 % after 12 h, while when pH=4.5, the drug release was just 58 % after 12 h. The KET-loaded liposomes appeared to release more rapidly, and also release a greater final cumulative release percentage, at the higher pH value. However, ketoprofen is an acidic drug, so it will be more soluble at pH 7.4 than at pH 4.5. In order to be sure that the difference is due to the liposomes rather than the inherent solubility of the drug, we prepared control particles made of polyvinylpyrrolidone (PVP), PC, and KET (see Supplementary Information, Fig S3). The drug release profiles of liposomes self-assembled from the PVP/PC/KET microparticles are given in Fig S4. It is clear that the release profile is very similar at both pH 4.5 and 7.4, with only a small increase in the release rate and final cumulative percent release reached at pH 7.4 (compared to pH 4.5). The difference between the profiles is much more pronounced in the case of F6, and hence it can be concluded that it is the presence of Eudragit that causes liposomes assembled from these particles to show pH-sensitive release.

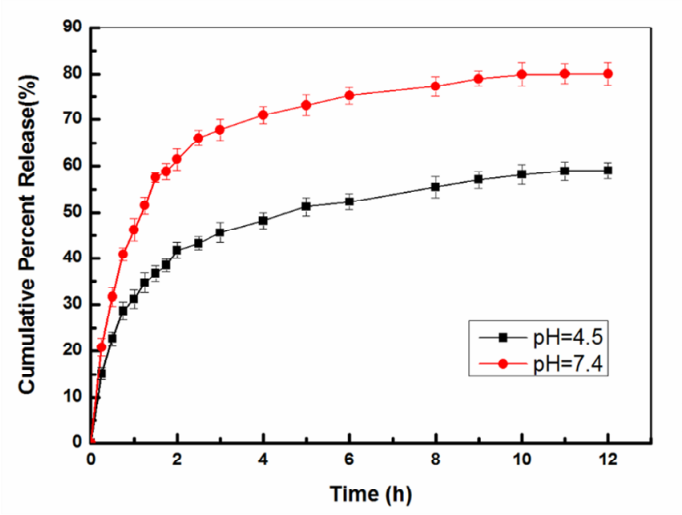

Figure. 6 The *in vitro* drug release profiles of liposomes obtained from F6, measured at pH=4.5 and pH=7.4.

The pH-responsive liposomes prepared in this work have a number of potential applications as targeted delivery systems. For example, in the human gastrointestinal tract, the pH value increases going from the stomach to the small intestine[43], which can be exploited to target release.

3.5 Proposed mechanism of liposome self-assembly and drug release

Based on the above results, it is clear that liposomes are easily formed from the Eudragit systems through self-assembly, and have pH-sensitive properties. A schematic diagram illustrating the proposed mechanism underlying this is shown in Fig 7. It is believed, on the basis of XRD and FTIR results, that the Eudragit and PC are mixed on the molecular level after electrospraying. This arises because the solvent evaporates very quickly, preventing the formation of a crystalline lattice, and instead hydrogen bonds form between the different components of the electrosprayed particles [35]. After the microparticles were dissolved in PBS, the Eudragit matrix dissolves quickly due to the ionization of its COOH groups to COO^-, while the insoluble PC and KET self-assemble into liposomes[30]. However, there will be electrostatic attractions between the negatively charged carboxyl groups in Eudragit and the positively charged quaternary ammonium group in PC. Thus, it is likely that the Eudragit chains can become anchored on the liposomes formed through electrostatic attractions. When the pH value of the liposomal solution is below 6.0, the Eudragit chains will become insoluble due to the protonation of

COO^- groups, and thus they will aggregate at the liposome exteriors, packing tightly to minimize Eudragit/water interactions. This is in accordance with the increase in size as seen at pH < 6, and will help to prevent drug release from the liposomes.

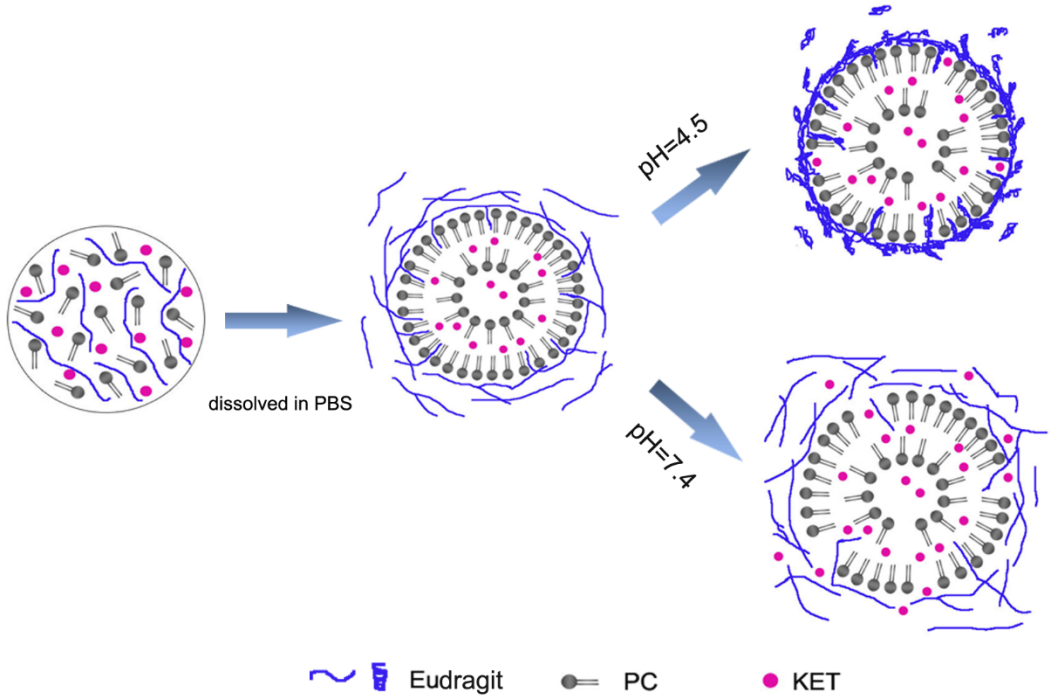

Figure. 7 A schematic diagram illustrating the self-assembly of liposomes from Eudragit/PC microparticles, and the effect of pH on the drug release.

When the pH of the liposomal suspension is higher than 6, the Eudragit chains will ionize and dissolve, either becoming freed into solution or attached loosely around the liposomes. Given the reduction in size seen at higher pH, we suspect that the former is the dominant process, although the TEM data at pH 7.4 indicate that some Eudragit molecules remain associated with the liposomes. These effects leave the KET inside the liposomes with a clear path to diffuse out into solution, promoting its release.

4. Conclusions

In this study, we report a simple method to fabricate pH-sensitive liposomes. Eudragit/phosphatidyl choline (PC) composite microparticles were first prepared by electrospraying. Analogous particles were

also prepared loaded with the model drug ketoprofen (KET). IR spectroscopy and X-ray diffraction data suggested that the components were mixed on the molecular level, while electron microscopy revealed the formation of spherical particles at around 1.2 – 1.6 μm in size. Liposomes could be self-assembled from the electrosprayed microparticles by dispersing them in a pH 7.4 phosphate buffer. Transmission electron micrographs suggested that these liposomes were roughly spherical in shape, with core/shell structures. The latter observation indicated the presence of Eudragit on the exterior of the PC core. In support of this idea, the resultant liposomes undergo distinct size changes when exposed to different pH environments. *In vitro* drug release experiments found that the liposomes had high entrapment efficiency for KET (*ca.* 75%) and can control the release of the incorporated KET in response to pH. Our findings indicate that the self-assembled pH-sensitive liposomes prepared in this work could have potential applications as advanced drug delivery systems.

5. Acknowledgements

This investigation was supported by grant 16410723700 from the Science and Technology Commission of Shanghai Municipality, the Biomedical Textile Materials “111 Project” of the Ministry of Education of China (No. B07024), and the UK-China Joint Laboratory for Therapeutic Textiles (based at Donghua University).

6. References

[1] O.A. Andreev, D.M. Engelman, Y.K. Reshetnyak, Targeting acidic diseased tissue: New technology based on use of the pH (Low) Insertion Peptide (pHLIP), Chimica oggi, 27 (2009) 34.

[2] W. Li, F. Nicol, F.C. Szoka, GALA: a designed synthetic pH-responsive amphipathic peptide with applications in drug and gene delivery, Advanced drug delivery reviews, 56 (2004) 967-985.

[3] E.A. Sosunov, E.P. Anyukhovsky, A.A. Sosunov, A. Moshnikova, D. Wijesinghe, D.M. Engelman, Y.K. Reshetnyak, O.A. Andreev, pH (low) insertion peptide (pHLIP) targets ischemic myocardium, Proceedings of the National Academy of Sciences, 110 (2013) 82-86.

[4] W.T. Al-Jamal, K. Kostarelos, Liposomes: from a clinically established drug delivery system to a nanoparticle platform for theranostic nanomedicine, Accounts of chemical research, 44 (2011) 1094-1104.

[5] B.T. Luk, R.H. Fang, L. Zhang, Lipid-and polymer-based nanostructures for cancer theranostics,

Theranostics, 2 (2012) 1117-1126.
[6] K. Ruckmani, V. Sankar, M. Sivakumar, Tissue distribution, pharmacokinetics and stability studies of zidovudine delivered by niosomes and proniosomes, Journal of Biomedical Nanotechnology, 6 (2010) 43-51.
[7] C.W. How, A. Rasedee, S. Manickam, R. Rosli, Tamoxifen-loaded nanostructured lipid carrier as a drug delivery system: characterization, stability assessment and cytotoxicity, Colloids & Surfaces B Biointerfaces, 112 (2013) 393-399.
[8] L. Yao, J. Daniels, D. Wijesinghe, O.A. Andreev, Y.K. Reshetnyak, pHLIP®-mediated delivery of PEGylated liposomes to cancer cells, Journal of Controlled Release, 167 (2013) 228-237.
[9] C.-J. Chu, F.C. Szoka, pH-sensitive liposomes, Journal of Liposome Research, 4 (1994) 361-395.
[10] B. Lin, C. Yin, H. Hauser, The effect of positive and negative pH-gradients on the stability of small unilamellar vesicles of negatively charged phospholipids, Biochimica et Biophysica Acta (BBA)-Biomembranes, 1147 (1993) 237-244.
[11] C. Fonseca, J.N. Moreira, C.J. Ciudad, M.C.P. de Lima, S. Simoes, Targeting of sterically stabilised pH-sensitive liposomes to human T-leukaemia cells, European journal of pharmaceutics and biopharmaceutics, 59 (2005) 359-366.
[12] S.-M. Lee, H. Chen, C.M. Dettmer, T.V. O'Halloran, S.T. Nguyen, Polymer-caged lipsomes: a pH-responsive delivery system with high stability, Journal of the American Chemical Society, 129 (2007) 15096-15097.
[13] S. Simões, J.N. Moreira, C. Fonseca, N. Düzgüneş, M.C.P. de Lima, On the formulation of pH-sensitive liposomes with long circulation times, Advanced drug delivery reviews, 56 (2004) 947-965.
[14] D. Jain, A.K. Panda, D.K. Majumdar, Eudragit S100 entrapped insulin microspheres for oral delivery, AAPS Pharmscitech, 6 (2005) E100-E107.
[15] Y. Krishnaiah, V. Satyanarayana, B.D. Kumar, R. Karthikeyan, In vitro drug release studies on guar gum-based colon targeted oral drug delivery systems of 5-fluorouracil, European journal of pharmaceutical sciences, 16 (2002) 185-192.
[16] U.E. Illangakoon, D.-G. Yu, B.S. Ahmad, N.P. Chatterton, G.R. Williams, 5-Fluorouracil loaded Eudragit fibers prepared by electrospinning, International journal of pharmaceutics, 495 (2015) 895-902.

[17] R.M. Straubinger, N. Düzgünes, D. Papahadjopoulos, pH-sensitive liposomes mediate cytoplasmic delivery of encapsulated macromolecules, FEBS letters, 179 (1985) 148-154.

[18] A. Catalan-Latorre, M. Ravaghi, M.L. Manca, C. Caddeo, F. Marongiu, G. Ennas, E. Escribano-Ferrer, J.E. Peris, O. Diez-Sales, A.M. Fadda, Freeze-dried eudragit-hyaluronan multicompartment liposomes to improve the intestinal bioavailability of curcumin, European Journal of Pharmaceutics and Biopharmaceutics, 107 (2016) 49-55.

[19] C.-c. Jin, H.-y. Li, G.R. Williams, R. Wei, H.-l. Nie, J. Quan, L.-m. Zhu, Self-assembled liposomes from electrosprayed polymer-based microparticles, Colloid and Polymer Science, 292 (2014) 2325-2334.

[20] D. Wang, H. Möhwald, Template-directed colloidal self-assembly–the route to 'top-down'nanochemical engineering, Journal of Materials Chemistry, 14 (2004) 459-468.

[21] Y. Yin, Y. Lu, B. Gates, Y. Xia, Template-assisted self-assembly: a practical route to complex aggregates of monodispersed colloids with well-defined sizes, shapes, and structures, Journal of the American Chemical Society, 123 (2001) 8718-8729.

[22] N. Stephanopoulos, J.H. Ortony, S.I. Stupp, Self-assembly for the synthesis of functional biomaterials, Acta materialia, 61 (2013) 912-930.

[23] A.V. Aggarwal, S.S. Jester, S.M. Taheri, S. Foerster, S. Hoeger, Molecular spoked wheels: synthesis and self‐assembly studies on rigid nanoscale 2D objects, Chemistry–A European Journal, 19 (2013) 4480-4495.

[24] D. Doty, L. Kari, B. Masson, Negative interactions in irreversible self-assembly, Algorithmica, 66 (2013) 153-172.

[25] S. Tanaka, A. Doi, T. Matsui, Y. Miyake, Mass transport and electrolyte accessibility through hexagonally ordered channels of self-assembled mesoporous carbons, Journal of Power Sources, 228 (2013) 24-31.

[26] C. Wang, Z. Wang, X. Zhang, Amphiphilic building blocks for self-assembly: from amphiphiles to supra-amphiphiles, Accounts of chemical research, 45 (2012) 608-618.

[27] Y. Wang, H. Xu, X. Zhang, Tuning the Amphiphilicity of Building Blocks: Controlled Self‐Assembly and Disassembly for Functional Supramolecular Materials, Advanced Materials, 21 (2009) 2849-2864.

[28] L. Zang, Y. Che, J.S. Moore, One-dimensional self-assembly of planar π-conjugated molecules:

adaptable building blocks for organic nanodevices, Accounts of chemical research, 41 (2008) 1596-1608.

[29] D.G. Yu, C. Branfordwhite, G.R. Williams, S.W.A. Bligh, K. White, L.M. Zhu, N.P. Chatterton, Self-assembled liposomes from amphiphilic electrospun nanofibers, Soft Matter, 7 (2011) 8239-8247.

[30] D.G. Yu, J.H. Yang, X. Wang, F. Tian, Liposomes self-assembled from electrosprayed composite microparticles, Nanotechnology, 23 (2012) 105606.

[31] D.G. Yu, L.M. Zhu, S.W. Bligh, C. Branford-White, K.N. White, Coaxial electrospinning with organic solvent for controlling the size of self-assembled nanoparticles, Chemical Communications, 47 (2011) 1216-1218.

[32] S. Vemuri, C.T. Rhodes, Preparation and characterization of liposomes as therapeutic delivery systems: a review, Pharmaceutica Acta Helvetiae, 26 (1995) 95-111.

[33] Z.M. Huang, Y.Z. Zhang, M. Kotaki, S. Ramakrishna, A review on polymer nanofibers by electrospinning and their applications in nanocomposites, Composites Science & Technology, 63 (2003) 2223-2253.

[34] M. Aceituno-Medina, A. Lopez-Rubio, S. Mendoza, J.M. Lagaron, Development of novel ultrathin structures based in amaranth (amaranthus hypochondriacus) protein isolate through electrospinning, Food Hydrocolloids, 31 (2013) 289-298.

[35] Y. Liu, G. Ma, D. Fang, J. Xu, H. Zhang, J. Nie, Effects of solution properties and electric field on the electrospinning of hyaluronic acid, Carbohydrate Polymers, 83 (2011) 1011-1015.

[36] J. Hu, H.Y. Li, G.R. Williams, H.H. Yang, L. Tao, L.M. Zhu, Electrospun poly(N-isopropylacrylamide)/ethyl cellulose nanofibers as thermoresponsive drug delivery systems, Journal of pharmaceutical sciences, 105 (2016) 1104-1112.

[37] H. Li, G.R. Williams, J. Wu, Y. Lv, X. Sun, H. Wu, L.M. Zhu, Thermosensitive nanofibers loaded with ciprofloxacin as antibacterial wound dressing materials, International Journal of Pharmaceutics, 517 (2016) 135.

[38] R.G. King, R.M. Marchbanks, The effect of pH on the size of liposomes formed by cholate dialysis, Biochimica Et Biophysica Acta, 691 (1982) 183-187.

[39] W. Zhou, X. An, J. Wang, W. Shen, Z. Chen, X. Wang, Characteristics, phase behavior and control release for copolymer–liposome with both pH and temperature sensitivities, Colloids & Surfaces A Physicochemical & Engineering Aspects, 395 (2012) 225-232.

[40] K. Khosravi‐Darani, M.E. Khoosfi, H. Hosseini, Encapsulation of zataria multiflora boiss. essential oil in liposome: antibacterial activity against E. Coli O157:H7 in broth media and minced beef, Journal of Food Safety, 36 (2016) 515-523.

[41] Y. Liao, L. Zhang, Y. Gao, Z.T. Zhu, H. Fong, Preparation, characterization, and encapsulation/release studies of a composite nanofiber mat electrospun from an emulsion containing poly (lactic-co-glycolic acid), Polymer, 49 (2008) 5294-5299.

[42] M.P.P. Maedeh Zamani, Seeram Ramakrishna, Advances in drug delivery via electrospun and electrosprayed nanomaterials, International Journal of Nanomedicine, 8 (2013) 2997-3017.

[43] M.Z. Khan, H.P. Stedul, N. Kurjaković, A pH-dependent colon-targeted oral drug delivery system using methacrylic acid copolymers. II. Manipulation of drug release using Eudragit L100 and Eudragit S100 combinations, Drug Development and Industrial Pharmacy, 26 (2000) 549-554.