International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 11-02-2019; Accepted: 14-03-2019
www.pharmacyjournal.in
Volume 4; Issue 3; May 2019; Page No. 29-34

Optimization of polycaprolactone nanoparticles by polyethylene glycol for protein delivery

Samuel Girgis[1*], Nazim Uddin[2]
[1, 2] School of pharmacy and pharmaceutical sciences, University of Sunderland University Department, Sunderland, State SR1 3SD, United Kingdom

Abstract
The aim of the current study is to design amphiphilic poly ε-caprolactone/polyethylene glycol nanoparticles (PCL/PEG) having better physicochemical properties than the PCL nanoparticles for protein delivery. Bovine serum albumin poly ε-caprolactone and polyε-caprolactone/polyethylene glycol nanoparticles were designed by the double emulsion/solvent evaporation method. Size was checked by photon correlation spectroscopy technique using Malvern zetasizer. Zeta potential wasmeasured by laser anemomerty technique using Malvern zetasizer. Protein loading efficiency and release rate were analysed by bicinchoninic acid assay using plate reader spectrophotometer. Morphology and size of the particles were displayed by scanning electron microscopy. All the (PCL/PEG) blends produce nanoparticles with smaller sizes, narrower PDI(s), and higher protein release rates than the BSA loaded LPCL nanoparticles. The low molecular weight polyethylene glycol (SPEG) blended (LPCL/SPEG2.5% and LPCL/SPEG5%) nanoparticles have higher protein loading efficiencies, smaller sizes, narrower PDI(s), and higher protein release rates than the BSA loaded LPCL nanoparticles.

Keywords: poly ε-caprolactone, polyethylene glycol, Bovine serum albumin, Double emulsion, Solvent evaporation

1. Introduction
Polycaprolactone (PCL) is a good candidate to design nanoparticles because its physical, chemical, and mechanical properties can be easily modified by copolymerization or blending or grafting with other polymers [1]. PCL is a hydrophobic polymer and reported to be compatible with various hydrophilic polymers like polyvinyl alcohol (PVA) and amphiphilic polymers like polyethylene glycol (PEG) [2]. The previous studies of PCL over the past 5-6 years were more dominant for the scaffold materials than formulations for drug delivery. The majority of previous PCL formulation studies included only its copolymerization with various polymers [1, 2].

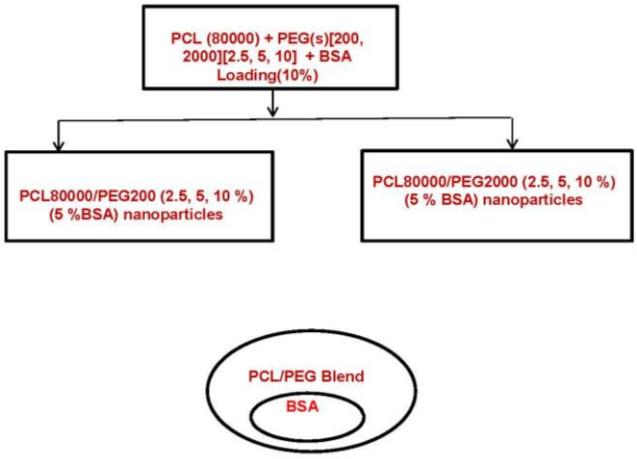

Fig 1: Project outline

2. Materials and Methods
2.1. Materials
All the chemicals were of analytical HPLC grade and used as purchased from source without any further purification. High molecular weight poly ε-caprolactone (LPCL) [80000], polyvinyl alcohol (PVA), low molecular weight polyethylene glycol (SPEG), high molecular weight polyethylene glycol (LPEG), dichloromethane (DCM), bovine serum albumin (BSA), potassium chloride (KCL), hydrochloric acid (HCL),). Phosphate buffer saline (PBS)and sodium hydroxide (NaOH), were purchased from (Aldrich, UK). bicinchoninic acid (BCA) and Cupper sulphate (CuSo4) were purchased from (Thermo-scientific).

2.2. Methodology
2.2.1. Preparation of the nanoparticles by double emulsion/solvent evaporation (DE/SE) technique.
PCL and PCL/PEG blend nanoparticles nanoparticles were prepared by a w/o/w solvent evaporation technique which encompassed the drop wise addition of 1ml of 2.5% m/v aqueous solution of polyvinyl alcohol (PVA) containing theoretical CK-10 loading of 1% m/m based on PLGA OR PLGA blend, to 8ml of dichloromethane (DCM) containing 100 mg of the polymer and different amounts of hydrophilic or amphiphilic polymer (5%) m/m, according to the experiment, based on the polymer, then homogenisation was done using the homogenizer (VWR VDI 25 Homogenizer, VWR international, UK) at a speed of 10000 revolutions per minute (rpm) for 2 minutes. The resulting w/o emulsion was subsequently added drop wise to 50ml of 1.25% m/v aqueous PVA solution followed by homogenising at a speed of 10K rpm using Silverson L4R homogeniser (Silverson, UK) for 6 minutes. The homogenisation process was performed over ice and the resulting w/o/w emulsion was stirred continuously and allowed to evaporate overnight. The particles were then collected by centrifugation (sigma centrifugation apparatus, Sigma, UK) at 4○C at 10K rpm for 30 minutes and washed with water a further 3 times using the same centrifugation parameters. The sediment obtained was resuspended in water and freeze dried at -85 0c & 0.012

mbar (Labconco freeze dryer, Freezone 4.5 Plus, Labconco, UK) for 48h [3-6].
Freeze drying of polymeric particles comprised the addition of diluted solutions of washed polymeric particles to 20 mL clear freeze-drying glass containers, covering with Parafilm M film perforated with 20 needle width holes, then allowing to freeze at -70oC for 2 hours. After that, the samples were lyophilised for 48 hours using the freeze dryer, under vacuum drawn by a high vacuum pump. Dried polymeric particles loaded with peptide were stored at room temperature in desiccator.

2.2.2 Determination of nanoparticle size by Malvern zetasizer using photon correlation spectroscopy (PCS) technique
Nanoparticles suspension was set by dispersing 5 mg of the nanoparticles in 2 ml distilled ddH2O, then 0.1 ml nanoparticles suspension were isolated in 3 to 4 ml of 0.22 µm filtered ddH2O, and characterized by Malvern instruments regarding polydispersity and Z-average diameter.

2.2.3 Measurement of zeta-potential of nanoparticles
Nanoparticles suspension was equipped by dispersing 5 mg of the nanoparticles in 2 ml distilled H20, and then 1 ml of the nanoparticle suspension was diluted in 0.001 M KCL, which works as a weak electrolyte, to get a sample of applicable concentration for the measurement.

2.2.4 Determination of bovine serum albumin amounts in nanoparticles by bicinchoninic acid (BCA) protein assay method
A 5mg nanoparticles sample was degraded in 1 ml of 1 M NaOH at 37°C overnight for digesting the polymer. The suspension was centrifuged and 25 µL of the supernatant was measured in 3 wells. A sequence of calibrated BSA protein standards were set in distilled water and 25 µL of each standard were added to 3 wells. 200 µL of BCA reagent were added to each well. Precision and accuracy of protein absorption were examined by using of a minimum of three absorption determinations for each standard, blank and test samples. After the purple colour progress, the absorbance of the contents of each well was identifiedat 562 nm using a plate reader.

2.2.5 Determination of protein release from polymeric nanoparticles
In vitro protein release from particles was achieved to define its release rate by incubation of 5mg of particles in 1 ml of phosphate buffered saline (PBS) having pH 7.4, containing 0.02% sodium azide as a bacteriostatic agent and 8 mM SDS in 1 ml eppendorf tubes. The particles were incubated at 37°C, and then samples were withdrawn at suitable time intervals, centrifuged and the quantity of BSA in the samples was evaluated using a BCA assay.

2.2.6 Scanning electron microscopy (SEM) for the determination of nanoparticles morphology
SEM microscopy is valuable to label the morphology and size of the nanoparticles. A tinny layer of nanoparticles was spread on a rounded aluminium plate using a carbon disc and the surface was then enclosed with a gold film using a sputter coater underneath an Argon atmosphere. Nanoparticles were identified by magnification with a scanning electron microscope (Cambridge Instruments Stereoscan 90B, 25 kV, Cambridge, UK) [7].

2.2.7. Transmission electron microscopy (TEM) for the determination of nanoparticles morphology

The TEM images were captured using a Philips EM 208S microscope working at various voltages. TEMs afford morphological, topographical, crystalline and compositional information for nano/microparticles [3]. The morphology of the PLGA and PLGA blends nanoparticles loaded with CK-10 were perceived, using transmission electron microscopy (TEM). One drop (100 µL) of the freshly-prepared nanoparticles suspension was deposited onto a glow-discharged carbon-coated electron microscopy grid. The excess liquid was detached by a piece of filter paper, and one drop of 2% uranyl acetate negative stain was added to the nanoparticle suspension before drying at room temperature [8].

2.2.8. Theory/calculation
The size of nanoparticles was determined by PCS technique using the Malvern Zetasizer nanoseries (Malvern Instruments, UK) which is useful for determination of the particle sizes of submicron particles in the range of 3 to 1000 nm. The theory of PCS technique is based on the use of dynamic light scattering for determination of the shape and size of particulate systems. The electric field of the incident light induces an oscillating polarisation on the particles in the sample, and then these particles whose polarity differs from the surroundings scatter the incident light. The particles in the sample are in constant motion which is called Brownian motion and this motion makes fluctuations in the detected intensity signal that can be measured digitally by PCS. The duration of the fluctuations provides information about the particles, including size and polydispersity [9, 10].
Zeta-potential was measured by laser anemometry in millivolts (mV) by using a Malvern zetasizer nanoseries. Zeta-potential is the electrical potential existing at the stern plane of a particle , which is an imaginary plane separating the thin layer of liquid bound to the particle surface from the rest of liquid and showing elastic and viscous behaviours. Colloidal particles are electrically charged due to their ionic characteristics and consequently, the distribution of ions in the neighbouring interfacial region will be affected by the resultant particle surface charge, and the counter ions (fixed layer) concentration will increase. A cloud- like area containing ions of opposite charges is formed outside the fixed layer. The net result is the formation of an electrical double layer in the region of the particle/liquid interface, with an inner region formed of ions strongly bound to the surface, and an outer diffuse ionic region. The potential in this region declines with the distance from the surface until at a certain distance it reaches zero [11, 12 & 13]. When a voltage is applied to the solution in which particles are suspended, particles are attracted to the electrode of the opposite polarity, associated by the fixed layer and part of the diffuse double layer. Nanoparticles suspension was prepared by dispersing 5 mg of the nanoparticles in 1 to 2 mL distilled H20, and then 0.5 mL of the nanoparticle suspension was diluted in 0.001 M KCl solution, which acts as a weak electrolyte, to get a sample of appropriate concentration for the measurement. LAT technique can also

be used to qualitatively check the adsorption of various targeting ligands on the surface NP(s) by showing the difference in zeta potential after adsorption of the targeting ligands.

The BCA protein assay includes the reduction of cupric (Cu2+) to cuprous (Cu1+) by protein in an alkaline medium tailed by a highly sensitive and selective colorimetric detection of the cuprous cation (Cu1+) by bicinchoninic acid [14].

The first step is the protein chelation for the copper in an alkaline medium to form a blue coloured complex (biuret reaction). The second step is the colour development reaction, in which BCA; a selective and sensitive colorimetric detection reagent reacts with the cuprous cation to form a purple coloured product by the chelation of one cuprous cation with two molecules of BCA. This purple coloured product is water soluble and can be measured spectrophotometrically at 562 nm. The Cu2+ reduction leading to BCA colour formation is highly affected by the presence of any of the four amino acid residues (tyrosine, tryptophan, cysteine and cystin) in the protein amino acid sequence. The peptide bond only is responsible for colour development at high temperatures and thus, the reaction is done at 60oC to increase the sensitivity. This technique has the advantages of compatibility with non-ionic and ionic detergents, minimum protein-to-protein variation and limited interactions with most copper chelators and reducing agents.

All results were subjected to statistical analysis using Microsoft excel 2010 software. All means were calculated as a mean ± standard error bar of the mean. All experiments were carried out in triplicate unless otherwise stated. Unpaired T tests was carried out on the data to check the significant difference when ($P < 0.05$) when compared to each other.

The ratios of PCL(s) amounts, PEG amounts and theoretical BSA loadings used for preparation of the various LPCL/PEG(s) nanoparticles are shown in table 1.

Table 1: The ratios of PCL(s) amounts, PEG amounts, and BSA loading.

LPCL (MWt 80000), mgs	PEG%(MWt)	Theoretical BSA Loading
200 mg	2.5% (PEG 200)	5
200 mg	5% (PEG 200)	5
200 mg	10% (PEG 200)	5
200 mg	2.5% (PEG 2000)	5
200 mg	5% (PEG 2000)	5
200 mg	10% (PEG 2000)	5

3. Results and Discussion: Table 2 shows the results of the LPCL/PEG(s) nanoparticles characterization which includes size (Z-average & PDI), zeta potential, actual loading and loading efficiency.

Table 2: Characterization of LPCL/PEG(s) nanoparticles.

Loaded polymer blend	Z- average ±s.d., nm	PDI± s.d.	Zeta potential ±s.d., mV	Theoretical Loading ,% m/m±s.d.	Actual Loading,%m/m± s.d.	Loading efficiency%±s.d.
LPCL	408 ± 11.79	0.51± 0.09	-10.02 ± 3.80	5	1.79 ± 0.35	35.73 ± 7.08
SPEG	242.47±	0.14±	-5.78±	5	2.71±	54.27±
2.5% /LPCL	20.02	0.01	0.31		0.29	9.76
SPEG5	254.67±	0.18±	-4.34±	5	1.97±	39.47±
% /LPCL	33.03	0.05	0.24		0.19	7.71
SPEG	269.20±	0.16±	-1.32±	5	1.49±	29.87±
10% /LPCL	30.44	0.02	0.16		0.18	5.56
LPEG	262.8	0.18±	-7.31±	5	1.43±1	28.53±
2.5% /LPCL	±14.86	0.02	0.63			6.01
LPEG5	299.43±	0.21±	-4.86±	5	1.38±	27.67±
% /LPCL	31.97	0.02	0.94		0.16	6.11
LPEG	332.67±	0.3±	-3.38±	5	1.08±	21.67±
10% /LPCL	29.50	0.08	0.50		0.08	4.52

The SPEG/LPCL nanoparticles show higher actual loading (2.71, 1.97, 1.49) than the LPEG/LPCL nanoparticles (1.43, 1.38, 1.08). On increasing the SPEG or LPEG in their blends, the actual loading decreases. It is important to notice that SPEG2.5%/LPCL and SPEG5%/LPCL nanoparticles have higher actual loadings than the 5% BSA loaded LPCL nanoparticles (1.79). Since the loading efficiency value is directly proportional to the actual loading, the SPEG/LPCL nanoparticles have higher loading efficiency (54.27, 39.47, 29. 87) than the LPEG/LPCL (28.53, 27.67, 21.67) and increasing the SPEG or LPEG in their blends decreases the loading efficiency. It is also obvious that SPEG2.5%/LPCL and SPEG5%/LPCL nanoparticles have higher loading efficiencies (54.27, 39.47) than the 5% BSA loaded LPCL nanoparticles (35.73).PEG is an amphiphilic polymer having hydrophobic and hydrophilic properties but the

polyesters like PCL are hydrophobic having a limited water-uptake. Proteins like BSA have a hydrophilic nature and are highly attached to hydrophilic moieties like PEG [15]. PEG can act as surfactant by accumulating at the inner interface of the particle consisting of the polymer mixture of polyesters and PEG to help protein spread throughout the inner phase [15]. However, the protein loading efficiency within the amphiphilic blend or copolymer of polyester and PEG is affected by the PEG MWt and amount. On blending small MWt PEG with polyester, water-uptake of the blend is low regardless of the polyester MWt but on increasing the PEG MWt, the water-uptake increases. The ''anti-water-uptake'' effect of the polyester becomes more pronounced with large MWt PEG due to the higher water uptake resulting in significant decrease of the protein loading [16]. Additionally, as the MWt of PEG(s) increases, the hydrophilicity power increases leading to protein release and diffusion through the matrix of the particles during the particles preparation resulting in decrease of the loading efficiency [17]. PEG can enhance protein loading within certain limits but on increasing the PEG content above these limits, water uptake by the matrix increases facilitating the protein diffusion towards the external aqueous phase during the particles preparation [18]. LPEG (2000) shows lower protein loading than SPEG (200) due to two reasons. Firstly, the LPEG has higher water uptake than the SPEG which enhances the prominence of the anti-water-uptake effect of the LPCL to decrease the BSA loading. Secondly, LPEG is more hydrophilic than the SPEG and consequently enhances the BSA d iffusion and release to the external aqueous phase during the DE/SE of LPCL/PEG nanoparticles.

Within the blends set of the SPEG/LPCL, SPEG has the highest loading at 2.5% as this amount is the optimum to promote the BSA diffusion towards the inner interface of the SPEG/LPCL nanoparticles but on increasing the SPEG content above 2.5%, water uptake by the SPEG/LPCL blend increases enhancing the partition and diffusion of the BSA towards the external aqueous phase. Regarding the LPEG/LPCL blends, they share the SPEG/LPCL blends the same phenomenon for the PEG content; as the LPEG content increases, the BSA loading decreases due to the same reason. It is important to note that each of the SPEG2.5%/LPCL and SPEG5%/LPCL nanoparticles achieve higher BSA loading than the 5% BSA loaded LPCL nanoparticles whereas the SPEG2.5% /LPCL nanoparticles have the highest loading efficiency than the other blends nanoparticles.

On comparing the effect of the two MWt PEG(s), it is observed that SPEG/LPCL blends produce nanoparticles with smaller sizes (242.47, 254.67, 269.2) and narrower PDI(s) (0.14, 0.18, 0.16) than LPEG/LPCL [sizes: (262.8, 299.43, 332.67), PDI(s) (0.18 0.21, 0.3) (fig 2).

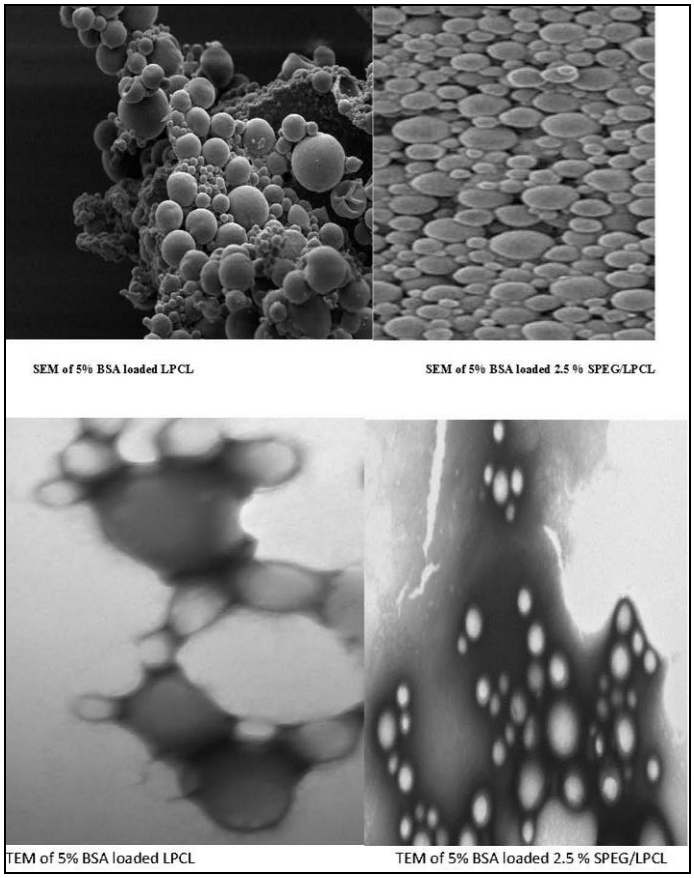

Fig 2: SEM & TEM images of the LPCL and 2.5% SPEG/LPCL

Moreover, increasing either the content of SPEG or LPEG in their blends increases the size and PDI of the nanoparticles. It is also clear that all the PEG/LPCL blends produce nanoparticles with smaller sizes and narrower PDI(s) than the 5% BSA loaded LPCL nanoparticles (408, 0.51).It was proved before that using surfactants like tween or PEG decreased the size and narrowed the PDI of the nanoparticles by producing uniform finer emulsion droplets through lowering the interfacial tension of the emulsion during the particles preparation [17, 18]. This fact can describe why all the LPCL/PEG nanoparticles have smaller sizes and narrower PDI(s) than the original 5% BSA loaded LPCL

nanoparticles. It was also reported that increasing the PEG MWt or content increases the water uptake and the polymeric aggregation to produce a more compact steric arrangement giving larger nanoparticles [19]. The LPEG produces larger nanoparticles with wider PDI than the SPEG due to its higher water uptake and entanglement than the SPEG. Nevertheless, increasing the PEG content in SPEG/LPCL and LPEG/LPCL blends increases the nanoparticles sizes and PDI(s) due to the enhancement of water uptake and PEG aggregation. According to the aforementioned fact that nanoparticles with lower sizes and narrow PDI(s) are preferred because these two factors increase the stability of nanoparticles in the emulsion [20, 21], therefore SPEG2.5%/LPCL nanoparticles are more stable in emulsion than the other blends nanoparticles due to their lowest sizes and narrowest PDI(s).

The SPEG is more effective in decreasing the zeta potential values than the LPEG. SPEG blends have lower zeta potentials (-5.78, -4.34, -1.32) than LPEG blends (- 7.31, -- 4.86, -3.38). Increasing the amount of either the SPEG or LPEG in their blends enhances the reduction of the zeta potential. It is also obvious that all the LPCL/PEG nanoparticles have lower zeta potentials than the 5% BSA loaded LPCL nanoparticles (- 10.02).Coating of nano/microparticles with many surfactants like amiphiphilic polymers normally reduces the zeta potential because the coating layers can mask the surface charge and move the shear plane outwards from the particle surface [20]. This proves that the surfactant molecules, at least in part are located on the surface of the particles, which consequently displace the shear plane of the diffuse layer producing lower zeta potential values (Santander-Ortega et al. 2007). Zeta potential of the particles is also affected by the amount the hydrophilic polymers used; increasing content of the hydrophilic polymers leads to more reduction of the zeta potential due to enhancement of the shielding effect of the hydrophilic polymers at the nanoparticle surface [21, 22]. All the LPCL/PEG nanoparticles are negatively charged due to the presence of charged carboxyl groups on the surface of the nanoparticles and presence of PEG in LPCL/PEG nanoparticles can be indicated by the lower zeta potential compared to LPCL nanoparticles. SPEG can be easier and more located on the surfaces of LPCL nanoparticle than LPEG due to its lower MWt, so the shielding effect of the SPEG on the nanoparticle surface is more pronounced than LPEG and as a result, SPEG/LPCL nanoparticles have lower zeta potential than LPEG/LPCL nanoparticles. Increasing the content of SPEG or LPEG in their blends increases the shielding effect at the nanoparticle surface that can obviously decreases the zeta potential. The SPEG2.5%/LPCL and LPEG2.5%/LPCL can have the best stability in suspensions because they have the highest zeta potentials.

PEG can increase the protein release rate when used as graft or copolymer or in blend with the polyesters by many mechanisms. The most important mechanism is increasing water absorption by its hydrophilic property that helps diffusion of the protein resulting in high release rate [15, 16, 17]. PEG may lead to pores formation at the surface of the polyester particles that enhances water penetration into the particles producing higher release of the protein. On increasing the MWt or the content of the PEG, the particles acquire more and more hydrophilic property which consequently increases water absorption leading to higher protein release. Since the LPEG has more oxyethlene content than the SPEG, therefore the LPEG blends have higher hydrophilic power for more water absorption that results to more protein diffusion and release than the SPEG blends. Increasing the amount of SPEG or LPEG in their blends also raises the hydrophilic property for more water absorption producing higher protein release.

4. Conclusions

Finally, it can be observed that the various PCL/PEG blends formulate nanoparticles having better physicochemical properties than LPCL nanoparticles. All the LPCL/SPEG and LPCL/LPEG blends produce nanoparticles with smaller size, narrower PDI, lower zeta potentials and higher protein release rates than the 5% BSA loaded LPCL nanoparticles. SPEG2.5%/LPCL and SPEG5%LPCL nanoparticles have higher protein loading efficiencies than the 5% BSA loaded LPCL nanoparticles.

We effectivelyverified the formulation and characterization of various PCL and amphiphilic PCL/PEG nanoparticles. Blending of PEG with PCL produces nanoparticles having better physicochemical properties than LPCL nanoparticles e.g. higher protein loading efficiencies, smaller sizes, narrower PDI(s), lower zeta potentials and higher protein release rates. All the PEG/LPCL nanoparticles have smaller sizes, narrower PDI(s), lower zeta potentials and higher protein release rates than the LPCL nanoparticles. The LPCL/SPEG2.5% and LPCL/SPEG5% nanoparticles achieve higher protein loading efficiencies than the LPCL nanoparticles.

These newly formed amphiphilic PCL/PEG nanoparticles system can be used as a good delivery system not only for proteins and peptides but also for hydrophilic and hydrophobic drugs. By increasing drug loading efficiency, large single dose can be administered instead of small frequent doses and by increasing the nanoparticles stability, the dose duration increases decreasing the administration frequency.

For better clearing of the benefits of this study, animal and human serum studies are recommended to be done on these various newly formed nanoparticles on loading different proteins like insulin, hydrophilic drugs like ranitidine and hydrophobic drugs like griseofulvin. Oral administration of certain proteins like insulin instead of injection will be more comfortable for the patients because it avoids infections, pain, contamination and other injection problems. This application will be highly valuable for treatment of diabetes because more than 347 million people worldwide have diabetes and a diabetic person has to pay direct costs for supplies and medication ranging from £ 621.5 to £ 9308 GBP a year. Moreover, it can enable the diabetic patients in the third world countries to avoid severe blood transmitted infections that occur on reusing of syringes e.g. AIDS and hepatitis c.

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5. Acknowledgments

Great cheers to the efforts of all the academic and technical staff for their efforts in the school of pharmacy, university of Sunderland

6. References

1. Dash TK, Konkimalla VB. Poly-ϵ-caprolactone based formulations for drug delivery and tissue engineering:

A review. Journal of Controlled Release. 2012; 158(1):15-33.
2. Woodruff MA, Hutmacher DW. The return of a forgotten polymer— Polycaprolactone in the 21st century. Progress in Polymer Science. 2010; 35(10):1217-1256.
3. Wang M, Thanou M. Targeting nanoparticles to cancer. Pharmacological Research. 2010; 62(2):90-99.
4. Wang AZ, Gu F, Zhang L, Chan JM, Radovic Moreno A, Shaikh MR, Farokhzad OC. Biofunctionalized targeted nanoparticles for therapeutic applications. Expert opinion on biological therapy. 2008; 8(8):1063-1070.
5. Masood F. Polymeric nanoparticles for targeted drug delivery system for cancer therapy. Materials Science and Engineering: C. 2016; 60:569-578.
6. Yoneki N, Takami T, Ito T, Anzai R, Fukuda K, Kinoshita, K, Sonotaki S, *et al*. One-pot facile preparation of PEG-modified PLGA nanoparticles: effects of PEG and PLGA on release properties of the particles. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2015; 469:66-72.
7. Gryparis EC, Hatziapostolou M, Papadimitriou E, Avgoustakis K. Anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles on LNCaP prostate cancer cells. European journal of pharmaceutics and biopharmaceutics. 2007; 67(1):1-8.
8. Chen H, Gao J, Lu Y, Kou G, Zhang H, Fan L, Sun Z, Guo Y, *et al*. Preparation and characterization of PE38KDEL-loaded anti-HER2 nanoparticles for targeted cancer therapy. Journal of Controlled Release. 2008; 128(3):209-216.
9. Khuroo T, Verma D, Talegaonkar S, Padhi S, Panda AK, Iqbal Z. Topotecan–tamoxifen duple PLGA polymeric nanoparticles: investigation of in vitro, in vivo and cellular uptake potential. International journal of pharmaceutics. 2014; 473(1-2):384-394.
10. Kocbek P, Obermajer N, Cegnar M, Kos J, Kristl J. Targeting cancer cells using PLGA nanoparticles surface modified with monoclonal antibody. Journal of controlled release. 2007; 120(1-2):18-26.
11. Martin Banderas L, Muñoz Rubio I, Prados J, Álvarez Fuentes, J, *et al*. In vitro and in vivo evaluation of Δ9-tetrahidrocannabinol/PLGA nanoparticles for cancer chemotherapy. International journal of pharmaceutics. 2015; 487(1-2):205-212.
12. Chan JM, Zhang L, Yuet KP, Liao G, Rhee JW, Langer R, Farokhzad OC. PLGA–lecithin–PEG core–shell nanoparticles for controlled drug delivery. Biomaterials. 2009; 30(8):1627-1634.
13. Jain AK, Thanki K, Jain S. Co-encapsulation of tamoxifen and quercetin in polymeric nanoparticles: implications on oral bioavailability, antitumor efficacy, and drug-induced toxicity. Molecular pharmaceutics. 2013; 10(9):3459-3474.
14. Bainor A, Chang L, McQuade TJ, Webb B, Gestwicki, JE. Bicinchoninic acid (BCA) assay in low volume. Analytical Biochemistry. 2011; 410(2):310-312.
15. Buske J, König C, Bassarab S, Lamprecht A, Mühlau S, Wagner KG. Influence of PEG in PEG–PLGA microspheres on particle properties and protein release. European Journal of Pharmaceutics and Biopharmaceutics. 2012; 81(1):57-63.
16. Tran V, Karam J, Garric X, Coudane J, Benoît J, Montero Menei CN, Venier Julienne M. Protein-loaded PLGA–PEG–PLGA microspheres: A tool for cell therapy. European Journal of Pharmaceutical Sciences. 2012; 45(1):128-137.
17. Bouillot P, Ubrich N, Sommer F, Minh Duc, T, Loeffler J, Dellacherie E. Protein encapsulation in biodegradable amphiphilic microspheres. International journal of pharmaceutics. 1999; 181(2):159-172.
18. Essa S, Rabanel JM, Hildgen P. Characterization of rhodamine loaded PEG-g-PLA nanoparticles (NPs): Effect of poly(ethylene glycol) grafting density. International journal of pharmaceutics. 2011; 411(1–2):178-187.
19. Adami R, Liparoti S, Izzo L, Pappalardo D, Reverchon E. PLA-PEG copolymers micronization by Supercritical Assisted Atomization. The Journal of Supercritical Fluids, 2012.
20. Sahoo SK, Panyam J, Prabha S, Labhasetwar V. Residual polyvinyl alcohol associated with poly (D, L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. Journal of Controlled Release. 2002; 82(1):105-114.
21. Santander Ortega M, Csaba, N, Alonso M, Ortega Vinuesa J, Bastos González D. Stability and physicochemical characteristics of PLGA, PLGA: poloxamer and PLGA: poloxamine blend nanoparticles: a comparative study. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2007; 296(1):132-140.
22. Dubey N, Varshney R, Shukla J, Ganeshpurkar A, Hazari PP, *et al*. Synthesis and evaluation of biodegradable PCL/PEG nanoparticles for neuroendocrine tumor targeted delivery of somatostatin analog. Drug delivery. 2012; 19(3):132-142.