International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685; Impact Factor: RJIF 5.28
Received: 07-12-2020; Accepted: 06-01-2021; Published: 10-02-2021
www.pharmacyjournal.net
Volume 6; Issue 1; 2021; Page No. 14-21

Design for optimization of novel ck-10 loaded nanoparticles

Girgis Samuel[1*], Uddin Nazim[2], Ankur Sharma[3], Veena Manuel[4]

[1] Lecturer of Phamaceutics and Drug Delivey, School of Phamacy, AlSalam University in Egypt (SUE), Egypt
[2] Assistant Professor, PhD in Pharmaceutical Sciences, Assistant Professor, University of Sunderland, England
[3] Department of Life Science, School of Basic Science and Research, Sharda University, India
[4] Research Project Manager, PMP®, CSPO®, Cancer Care Manitoba, Canada

Abstract
Nano carriers (NCs) have been established as drug carriers to be a gifted targeted drug delivery systems for cancer therapy. The physicochemical properties of NCs can be modified for improvements in the pharmacokinetic and pharma co-dynamic profiles for practical uses.CK-10 is a novel synthetic peptide that can significantly inhibit the RAN overexpression in malignant tissue and therefore it can successfully prevents the metastatic growth. However, CK10 still suffers from the traditional practical hurdles of the other peptides like poor stability, short half-life, and liability to digestion by proteases. Loading the CK-10 within various PLGA nanoparticles has the potential to solve such problems for treating cancer. Design of a novel PLGA nanoparticle by a novel microfluidics techniques is sufficient to optimize physicochemical properties. The novel microfluidic technique has succeeded to improve the physicochemical properties all the PLGA nanoparticles by enhancement of the peptide loading, decreasing the size and narrowing the polydispersity index compared to the double emulsion/solvent evaporation technique. PLGA/Poloxomer and PLGA/B cyclo dextrin formed by the novel microfluidic technique have higher CK-10 loading efficiencices (56.13 & 52.92) with suitable size range (200 to 260) for targeting the cancer tissues. PLGA/Poloxomer blend has the smallest size which is a vital parameter for targeting the cancer/tumor tissue. The successful development of better physicochemical properties for the CK-10 loaded PLGA nanoparticles can improve the RAN blocking by CK-10.

Keywords: CK-10, PLGA, microfluidics, solvent evaporation

Introduction

Sub micro metre or nano particulate structures can be a stage advancing towards enhanced drug delivery [1, 2]. The variance in dimensions between micro particles (MP) and nanoparticles (NP) has profound effects. The lower the particle size, the better the proportion of drug that can be encapsulated within the NP(s). Size has a clear effect on the destiny of particles injected into the vasculature. Micro particles can easily cause embolism to the vessels, which occludes blood flow, with or without drug delivery. This disadvantage can be avoided by using nanoparticles that are too small to produce embolism and can circulate through the vasculature [1].

The achievement of any therapeutic treatment relies not only on the pharmacokinetic and pharma co dynamic parameters of the pharmaceutical active agent, but also to a large extent on the bioavailability to the site of action in the organs. PLGA is an extensively used polymer for manufacturing NPs due to its biocompatibility, long-established track record in biomedical applications and good-documented efficacy for sustained drug release in comparison to the conventional systems up to months, and feasibility of parenteral administration by injection. Macromolecular drugs such as proteins, peptides, vaccines, antigens, genes, and human growth factors, are successfully encapsulated or loaded into PLGA or PLGA-based NPs [3, 7]. Several anticancer drugs like doxorubicin and 5-fluorouracil have been formulated using PLGA. In1999, the US FDA approved PLGA microsphere formulation, Nutro pin Depot, as a once-a month substitute to daily injections of human growth hormone.

Particle size is a vital aspect in the bio distribution of NPs and in attaining therapeutic efficacy. Small PLGA NPs have lower adsorption of plasma protein on their surface and also have lower hepatic filtration [5, 6]. Generally, delivery of NP for anticancer drugs to cancerous or tumor tissues can be accomplished by either passive or active targeting. Passive targeting depends on the advantage of the inherent NPs size and the unique anatomical and pathophysiological abnormalities of cancerous/tumor vasculature, such as the enhanced permeability and retention (EPR) effect (fig.1).

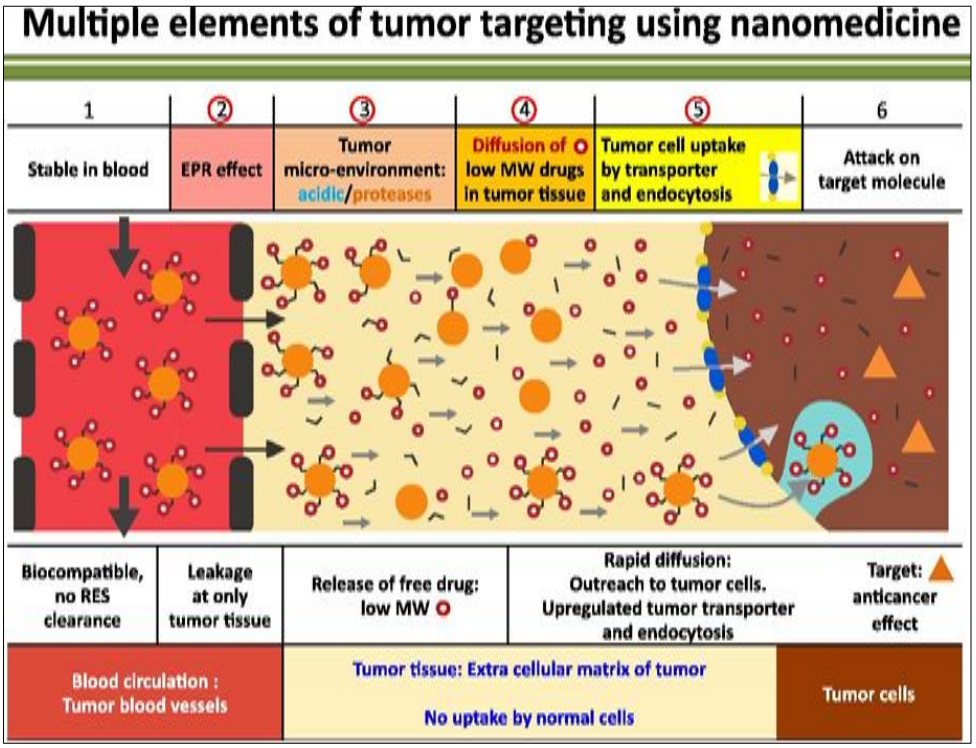


Fig 1: Multiple elements showing the importance of the EPR effect for the NP(s) uptake. Adapted from [3].

Materials and Methodology

All the chemicals were purchased from Sigma Aldrich UK. The novel CK-10 peptide was purchased from GL Bio chem in China where it was made by custom synthesis.

Preparation of the nanoparticles by double emulsion/solvent evaporation (DE/SE) technique.

1 mg CK-10 or 1mg CK-10 and 5 mg of the amphiphilic polymer were dissolved in 1 ml of internal aqueous phase (2.5 % W/V PVA) and mixed with 5 ml dichloromethane (DCM) containing 20 % w/v polymer, then emulsified at 10000 rpm (Silverson L5T, Silverson Machines, UK) for 2 min. The primary emulsion (w/o) was inoculated directly into a 1.25% w/v PVA solution under emulsification at 10,000 rpm for a further 6 min to produce a double emulsion and then centrifuged to form NP(s) pellet The pellet was washed three times with distilled water and lyophilised at -85 0c & 0.012 mbar (Lab conco freeze dryer, Free zone 4.5 Plus, UK) for 48 h [1, 7].

Preparation of the nanoparticles by microfluidics technique

The NPs of PLGA and PLGA blended polymers were prepared by a novel microfluidic technique (Nano Assemblr TM Bench top Instrument; Precision Nano Systems, Ltd., Vancouver, BC). 4 ml of the acetonitrile solution of PLGA 30000-60000 (25 % w/v) and 1 ml aqueous phase (2.5 % W/V PVA) for 1 mg CK-10 or 1 mg CK-10 and 5 mg of the amphiphilic polymer were mixed in the microfluidic mixing chamber at a flow rate of (12mL/min) by syringes indicated in the Nano Assemblr TM software. The NP(s) were recovered by salting out using a 20 ml K2HPO4 (150 mm) that could promote the phase separation of ACN–water more effectively under 4 ◦C at 15000 rpm for 30 minutes (sigma centrifugation apparatus, Sigma, UK) to pellet the NP(s) [7]. The pellet was washed and dried in similar conditions to the DE/SE.

Determination of CK-10 amounts in nanoparticles by Modified Lowry Protein assay method.

The peptide loading was estimated by straight extraction from lyophilised NP by solvent dissolution using dimethylsulfoxide (DMSO). Freeze-dried NP (5 mg) were weighed, dissolved in 0.5 ml DMSO and added to 0.5 ml solution of 0.1 N NaOH containing 0.5% SDS. The peptide concentration was analysed by the microplate Lowry assay to give the loading efficiency percentage [18, 20].

DeterminationofnanoparticlesizebyTunableporeresistive sensingtechnique (TPRS).

The NP(s) size was measured by using Izon q Nano particle sizer (Izon Science Ltd. New Zealand) using trizma buffer. The reference calibration particles (CPC) were chosen on the basis of predictable size of formulation particles and then suspended in trizma buffer supplied by Izon Company which was filtered by syringe & syringe filter 0.2 µm and put into Izon qnano using micro pipette [8].

Determination of nanoparticle zeta potential by Tunable pore resistive sensing technique (TPRS).

The surface charge measurements were established similarly to size analysis experiments, but with the investigated samples loaded into both halves of the fluid cell. Pressure was applied across the membrane using a sensitive (±9.8 Pa or 1 mm H2O) customized manometer [9]. During the experiment, the overpressure in the lower half of the fluid cell P = P1-P2 was continuously varied between ±490 Pa (±50 mm H2O).

Determination of nanoparticle size by photon correlation spectroscopy (PCS)

The nanoparticles suspension was prepared by dispersing 5mg of the nanoparticles in 1 to 2ml distilled ddH2O, then 0.1 ml nanoparticles suspension were dispersed in 2 to 3 ml of 0.22 µm filtered ddH2O, and then measured by Malvern

instruments with regard to poly dis persity and Z-average diameter [1, 7].

Measurement of zeta-potential of nanoparticles by laser anemometry technique

The nanoparticles suspension was prepared by dispersing 5mg of the nanoparticles in 1 to 2ml distilledH20, 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 [1-7].

Scanning electron microscopy (SEM) for the determination of Nano particles morphology

SEM microscopy is useful to describe the morphology and size of the Nano micro particles. A tinny layer of CK 10 NP(s) was spread on a rounded Aluminium plate using a carbon disc and the surface was then covered with a gold film using a sputter coater underneath an Argonatmosphere.NP(s) were recognized by magnification with a scanning electron microscope (Cambridge Instruments Stereoscan90B, 25kV, Cambridge, UK) [10].

Transmission electron microscopy (TEM) for the determination of

The morphology of the PLGA and PLGA blends nanoparticles loaded with CK-10 were observed, using transmission electron microscopy (TEM). One drop (100µL) of the freshly-prepared nanoparticles suspension was deposited onto a glow-discharged carbon-coated electron micro scopy grid. The excess liquid was removed 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 [11].

Results and discussion

The nanoparticles were prepared by the technique DE/SE and modified microfluidic technique.

The NP(s) were characterized by the same methods under the same conditions. The ratios of PLGA amounts, hydrophilic and amphiphilic polymers amounts and theoretical CK10 loadings used for preparation of the various nanoparticles are shown in table 1.

Table 1: The ratios of PLGA & PLGA/blend(s) amounts and CK-10 loading

Polymersused	Theoretical CK-10 loading
PLGA	1 %
PLGA/Chitosan5%	1 %
PLGA/PEG5%	1 %
PLGA/B cyclodextrin 5%	1 %
PLGA/Poloxomer5%	1 %
PLGA/PA 5%	1 %
PLGA/PVP 5%	1 %
PLGA/HPMA 5%	1 %
PLGA/PEI5%	1 %

*DE/SE: double emulsion/solvent evaporation. N: microfluidic Nano Assemblr

Loading efficiency

Aliphatic polyesters like PLGA have sufficient mechanical strength to carry a diversity of drug classes such as micro molecules, peptides and proteins [12, 13, 14].

The loading efficiency is influenced by numerous factors like the nature of the polymer, the Mwt of the polymer, the surfactant type, polymer/surfactant ratio, and additional hydrophilic and/or Amphiphilic polymer and peptide or protein concentration.

The polymer concentration in the organic phase adjusts the emulsion viscosity which can enhance the protein or peptide loading efficiency and prevent its diffusion towards the external aqueous phase [14, 15, 16].

All the PLGA blends show higher loading efficiency than the original PLGA in both of the techniques (DE/SE) & microfluidics ($P < 0.05$) (table 2).

Table 2: Loading efficiency of the nanoparticles

Polymeric nanoparticles	Actual Loading,%m/m, n=3, ± s.d.	Loadingefficiency%±, n=3, s.d.
PLGA-DE/SE	25.8±3.52	25.8±3.52
PLGA-N	37.14±5.14	37.14±5.14
PLGA chitosan-DE/SE	42.88±2.51	42.88±2.51
PLGA β-Cyclodextrin-D	43.88±2.4	43.88±2.4
PLGA β-Cyclodextrin,-N	53.92±7.95	53.92±7.95
PLGA PEG-DE/SE	38.6±5.81	38.6±5.81
PLGA PEG-N	48.65±9.09	48.65±9.09
PLGA PEI-DE/SE	34.89±1.85	34.89±1.85
PLGA PEIN	45.29±2.19	45.29±2.19
PLGA poloxamer-DE/SE	46.53±7.43	46.53±7.43
PLGA poloxamer-N	56.13±5.68	56.13±5.68
PLGA PA-DE/SE	45.07±2.84	45.07±2.84
PLGA PA-N	53.41±6.9	53.41±6.9
PLGA PVP-DE/SE	39.86±6.46	39.86±6.46
PLGA PVP-N	45.18±6.32	45.18±6.32
PLGA HPMA-DE/SE	34.86±4.46	34.86±4.46
PLGA HPMA-N	40.19±3.64	40.19±3.64

*DE/SE: double emulsion/solvent evaporation. N: microfluidic Nano Assemblr

The application of the Nano Assemblr shows higher loading efficiency than the DE/SE for all the nano particles whether the PLGA alone or the PLGA blends ($P < 0.05$). For instance, in the first stage of the w/o/w emulsion formulation process, the peptide dissolved in the aqueous phase might be denatured at the water /organic interface or unfold or aggregate due to the shear stress needed for the formation of the primary emulsion [17, 18]. The stability of the loaded proteins or peptides within the polyesters polymeric nano particles depends on many factors like the process used for

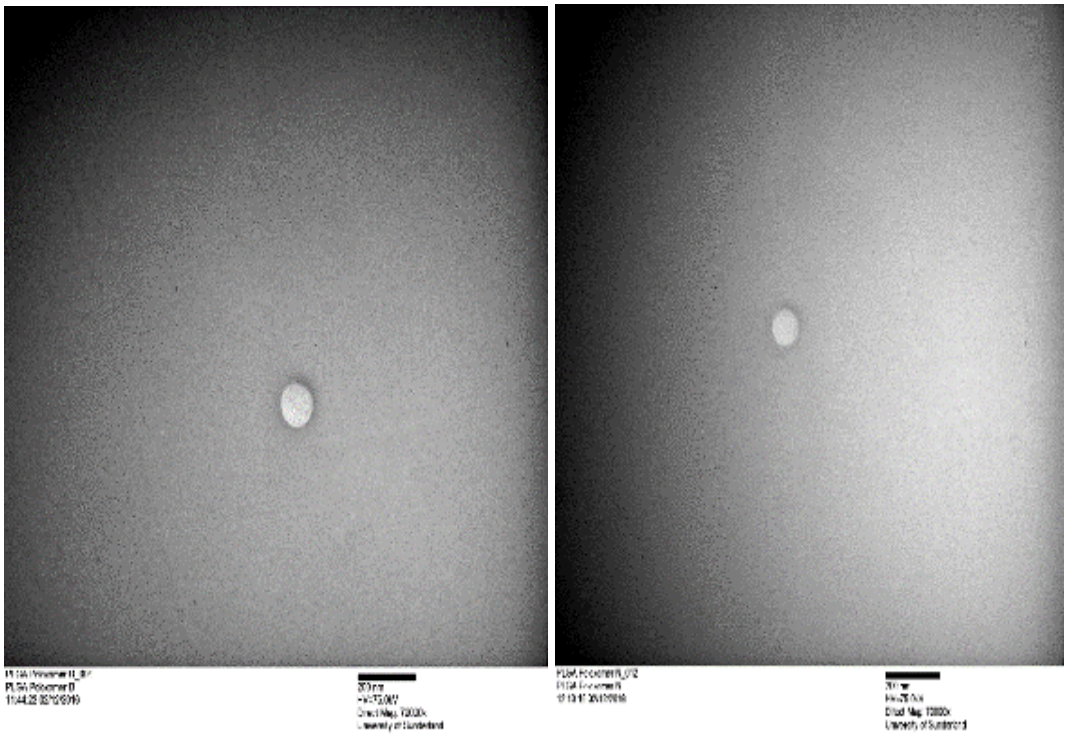
the nanoparticles formulation, characters of the used surfactants, and the properties of the polymer blends. Peptides like CK- 10 have a hydrophilic nature and can be highly attached to hydrophilic moieties of the hydrophilic or amphiphilic polymers [18].

This is the mechanism for describing the loading efficiency enhancement in all the used blends. Amphiphilic polymers (PEG, PVP, PoloXamers) have extra mechanism for improving the CK-10 loading such that they can also act as surfactants by accumulating at the internal interface of the particle consisting of the polymer mixture of polyesters and amphiphilic polymers to help the peptide spread throughout the innermost phase [19]. The application of the Microlfuidic mixing techniques is unique for the microfluidic devices, so it enables the establishment of organic polymeric nanoparticles in continuous flow higher drug loadings relative to those made with conventional emulsification technique.

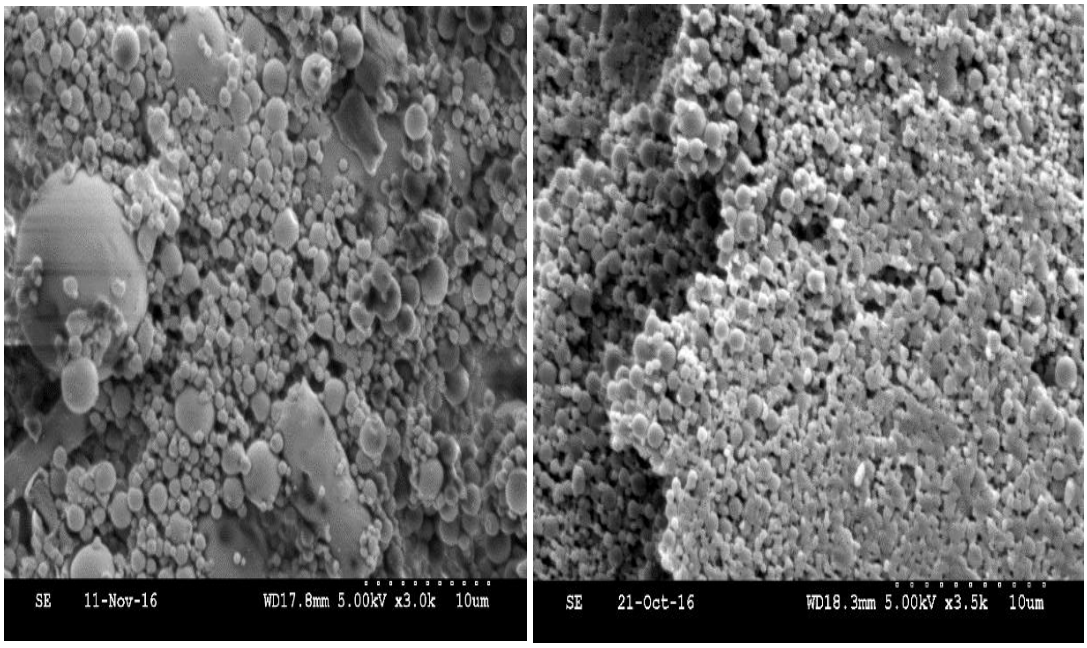
The nano assemblr applies the hydrodynamic flow procedure in few seconds. Under rapid microfluidic mixing using the nanoassemblr, solvent exchange is comprehensive even before the polymers commence aggregation [13-23].

Size

The use of the nanoassemlbr decreases the size ranges of all the nanoparticles ($P < 0.05$).



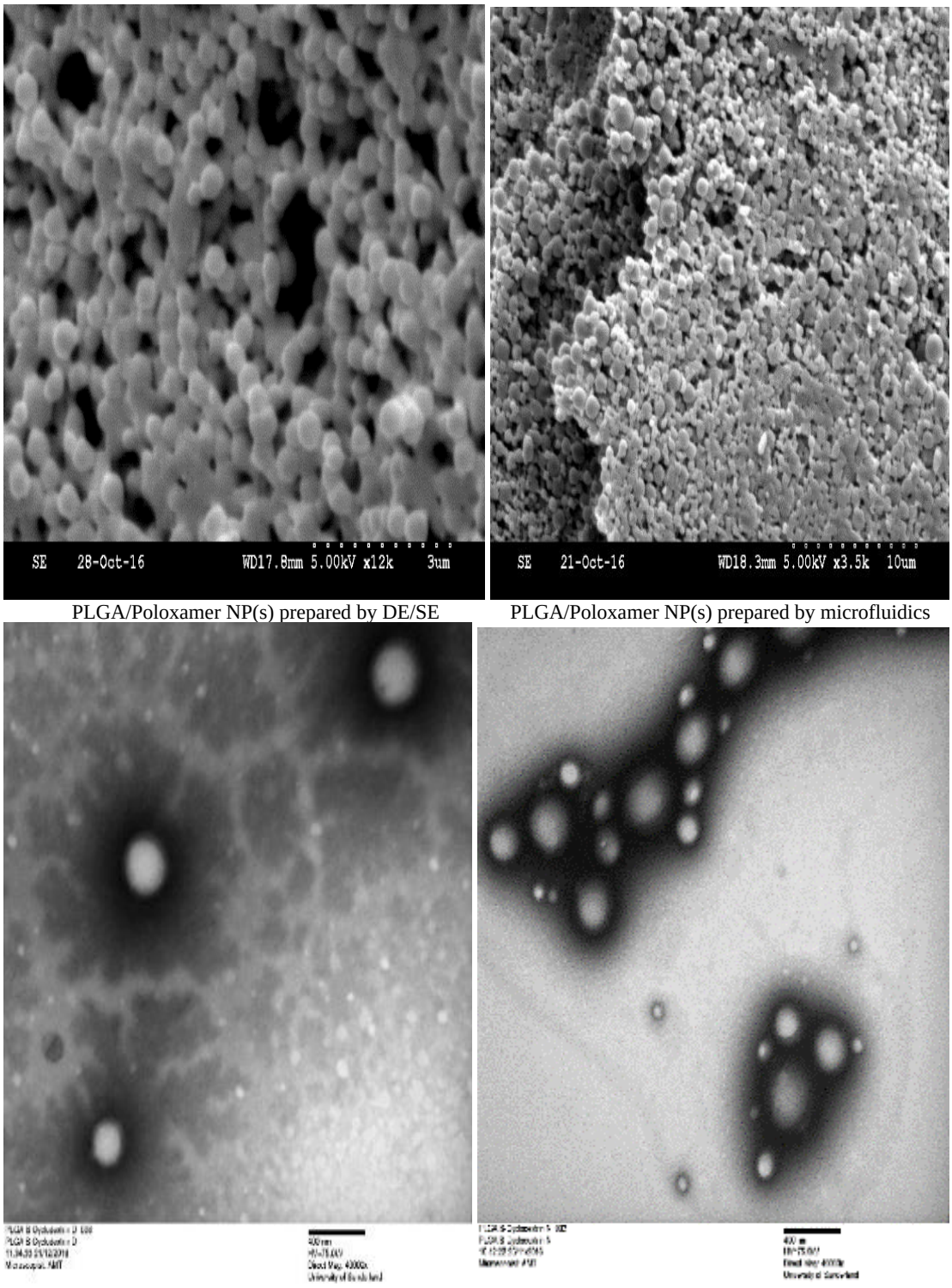
PLGA/PEG PLGA/PEG NP(s) NP(s) prepared by DE/SE prepared by Microfluidics



PLGA/B cyclodextrin NP(s) prepared by DE/SE PLGA/B cyclodextrinNP(s) prepared by microfluidics

Fig 2: TEM images of CK 10 PLGA/PEG NP(s) and SEM images of CK 10 PLGA/ B cyclodextrin NP(s)

However, the hydrophilic polymers/PLGA blends have larger sizes than the PLGA while the Amphiphilic polymers/PLGA have smaller sizes than the PLGA for both of the used techniques. The use of nano assemblr doesn't make significant differences for all the nano particles because each blend has closely related values in both techniques (fig.2, 3).



PLGA/Poloxamer NP(s) prepared by DE/SE

PLGA/Poloxamer NP(s) prepared by microfluidics

PLGA/B P NP(s) prepared by DE/SE

PLGA/B cyclodextrinNP(s) prepared by microfluidics

Fig 3: SEM images of CK 10 PLGA/Poloxamer NP(s) & TEM images of CK 10 PLGA/ B cyclodextrin NP(s)

The size of various polyester nanoparticles is affected by some factors like amount of the loaded drug, polymer/surfactant ratio, MWt of the polymer, physicochemical properties of any additional polymer and method of nanoparticles formulation [16, 22].

Table 3: Size of the nanoparticles by TRPS technique

Polymeric nanoparticles	Z-average± S.D.,nm
PLGA-DE/SE	254.33± 12.70
PLGA-N	238.00± 7.81
PLGA chitosan-DE/SE	263.00± 11.53
PLGA B Cyclodextrin-D	273.67± 8.50
PLGA B Cyclodextrin-N	254.33± 4.93
PLGA PEG-DE/SE	229.00± 10.00
PLGA PEG-N	214.67± 4.04
PLGA PEI-DE/SE	275.00± 6.00
PLGA PEIN	264.67±5.51
PLGA poloxomer-DE/SE	207.67± 5.69
PLGA poloxomer-N	196.00± 5.57
PLGA PA-DE/SE	269.67± 4.51
PLGA PA-N	255.00± 7.94
PLGA PVP-DE/SE	235.33± 6.11
PLGA PVP-N	219.00± 4.58
PLGA HPMA-DE/SE	268.00± 4.58
PLGA HPMA-DE/SE	253.33± 7.09

*DE/SE: double emulsion/solvent evaporation. N: microfluidic nanoassemblr.

It was also reported that the hydrophilic polymers could increase the water uptake, have a polymeric aggregation character to produce a more compact steric arrangement giving larger nanoparticles and lead to widening of the PDI [18, 19 & 20].

Generally, DLS technique shows non-significant overestimation (P > 0.05) for characterizing the size of the particles over than the TRPs technique because it lacks the several complicated factors used in the TRPS technique like the nanopore membrane, pressure and stable electric current.

Table 4: Size& PDI of the nanoparticles by DLS technique

Polymeric nanoparticles	Z-average± S.D.,nm	PDI± S.D.
PLGA-DE/SE	259.33 ± 2.68	0.26 ± 0.02
PLGA-N	245.40 ± 10.10	0.18 ± 0.06
PLGA chitosan-DE/SE	270.77 ± 6.67	0.28 ± 0.02
PLGA B Cyclodextrin-D	278.23 ± 3.21	0.34 ± 0.03
PLGA B Cyclodextrin-N	256.67 ± 2.60	0.27 ± 0.05
PLGA PEG-DE/SE	238.20 ± 5.56	0.24 ± 0.03
PLGA PEG-N	220.83 ± 10.40	0.17 ± 0.04
PLGA PEI-DE/SE	283.40 ± 6.07	0.33 ± 0.02
PLGA PEIN	270.70 ± 6.20	0.29 ± 0.02
PLGA poloxomer-DE/SE	215.70 ± 4.80	0.14 ± 0.01
PLGA poloxomer-N	208.90 ± 2.75	0.11 ± 0.01
PLGA PA-DE/SE	277.83 ± 4.51	0.38 ± 0.01
PLGA PA-N	261.97 ± 6.34	0.30 ± 0.08
PLGA PVP-DE/SE	240.90 ± 1.91	0.22 ± 0.07
PLGA PVP-N	227.97 ± 3.30	0.20 ± 0.01
PLGA HPMA-DE/SE	276.97 ± 5.02	0.41 ± 0.04
PLGA HPMA-N	260.37 ± 8.30	0.28 ± 0.06

*DE/SE: double emulsion/solvent evaporation. N: microfluidic nanoassemblr.

The DLS technique shows non-significant overestimation (P > 0.05) for the size analysis compared to the TRPS technique. Similarly, the use of the nano assemlbr decreases the size ranges of all the nanoparticles than the DE/SE technique.

The microfluidic production of micro droplets has lately been investigated extensively [13-18].

Nanoparticle production takes place in the micro droplets to increase the mixing efficiency in the microfluidic channels, and further shrinks the particle size distributions.

Zeta potential

All the hydrophilic and amphiphilic blends have lower zeta potential values than the original PLGA except the PLGA/PA such that it has the dramatic highest zeta potential in both of the used techniques (- 65.8 for DE/SE and -59.87 for hydrodynamic nano assemblr flow) (P< 0.05) (table 5).

Table 5: Zeta potential characterization of the various CK-10 loaded NP(s).

polymeric nanoparticles	Zeta-potential±s.d.,mV, by TRPS technique	Zeta-potential ± s.d., mV, by LAT technique
PLGA-DE/SE	-53.27± -4.96	-60.67 ± -7.85
PLGA-N	-51.97±-1.69	-57.77 ± -7.27
PLGA chitosan DE/SE	-36.23 ± -3.55	- 40.70 ± -4.81
PLGA B cyclodextrin-DE/SE	-45.03± -3.13	-51.07 ± -3.21
PLGA B cyclodextrin-N	-43.00± -2.43	-47.07 ± -4.61

PLGA PEG-DE/SE	-46.57± -2.15	-51.73 ± -4.92
PLGA PEG-N	-43.33± -2.01	-47.47 ± -6.56
PLGA PEI-DE/SE	-41.47± -5.68	-44.80 ± -5.85
PLGA PEI-N	-35.20± -3.92	-39.50 ± -1.48
PLGA poloxomer-DE/SE	-40.50± -2.25	-43.50 ± -4.92
PLGA poloxomer-N	-39.97± -6.67	-41.30 ± -4.70
PLGA PA-DE/SE	-65.80± -4.03	-66.80 ± -6.17
PLGA PA-N	-59.87± -4.22	--60.80 ± -6.17
PLGA PVP-DE/SE	-44.30± -4.55	-50.33 ± -0.64
PLGA PVP-N	-41.50± -3.40	-44.90 ± -6.29
PLGA HPMA-DE/SE	-43.90± -2.00	-50.00 ± -5.21
PLGA HPMA-N	-40.13± -3.34	-42.90 ± -4.71

*DE/SE: double emulsion/solvent evaporation. N: microfluidic nanoassemblr

The laser anemometry technique displays non-significant overestimation for the zeta potential of the nanoparticles compared to the TRPS ($P > 0.05$). The microfluidic nano assemblr nanoparticles don't have significant differences ($P > 0.05$) compared to the DE/SE nanoparticles because each blend has closely related values in both techniques with minor drop of the zeta potential for the microfluidic nano assemlr technique.

Upon adding PVA to the polyester, it constructs a layer at the polyester nanoparticles surface that can reasonably shield the negative charge of the polyesters nanoparticles surface in an amount dependent manner [22, 24]. Covering of nanoparticles with various amphiphilic or hydrophilic polymers normally decreases the zeta potential because the coating layers can disguise the surface charge and move the shear plane outwards from the particle surface [19, 24].

Conclusion

We successfully verified the formulation and characterization of several PLGA nanoparticles to check the optimization of the novel CK 10 NP(s). Blending of hydrophilic and amphiphilic polymers with PLGA produces nanoparticles having better physicochemical properties than PLGA nanoparticles, especially by using the novel microfluidic technique which can overcome several problems of the conventional techniques like the DE/SE e.g.higher peptide loading efficiencies, smaller sizes and higher uniformity.

References

1. Barik A, Choudhury IN, Chakravorty N. Parameter Optimization of Injectable Polycaprolactone Microspheres Containing Curcumin Using Response Surface Methodology. In 2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). 2018; 147-150 IEEE.
2. Khan J, Alexander A, Saraf S, Saraf S. Exploring the role of polymeric conjugates toward anti-cancer drug delivery. Curren trends and future projections. International journal of pharmaceutics, 2018.
3. Maeda H. Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. Advanced drug delivery reviews. 2015; 91:3-6.
4. Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A. *et al.* Margolese RG, Hoehn JL, Vogel VG, Dakhil SR, Tamkus D. Preoperative chemotherapy: updates of national surgical adjuvant breast and bowel project protocols B-18 and B-27. Journal of Clinical Oncology. 2008; 26(5):778-785.
5. Bilati U, Allémann E, Doelker E. Poly (D, L-lactide-co-glycolide) protein-loaded nanoparticles prepared by the double emulsion method-processing and formulation issues for enhanced entrapment efficiency. Journal of microencapsulation. 2005; 22(2):205-214.
6. Jung T, Breitenbach A, Kissel T. Sulfobutylated poly (vinyl alcohol)-graft-poly (lactide-co-glycolide) s facilitate the preparation of small negatively charged biodegradable nanospheres. Journal of controlled release. 2000; 67(2-3):157-169.
7. Lane RS, Haller FM, Chavaroche AA, Almond A, De Angelis PL. Heparosan-coated liposomes for drug delivery. Glycobiology. 2017; 27(11):1062-1074.
8. Lindeboom N, Wanasundara PKJPD. Interference of phenolic compounds in Brassica napus, Brassica rapa and Sinapis alba seed extracts with the Lowry protein assay. Food chemistry. 2007; 104(1):30-38.
9. Somerville JA, Willmott GR, Eldridge J, Griffiths M, Mc Grath KM. Size and charge characterisation of a sub micrometre oil-in-wa ter emulsion using resistive pulse sensing with tunable pores. Journal of colloid and interface science. 2013; 394:243-251.
10. Jaidev LR, Krishnan UM, Sethuraman S. Gemcitabine loaded biodegradable PLGA nanospheres for *in vitro* pancreatic cancer therapy. Materials Science and Engineering. 2015; 47:40-47.
11. Khuroo T, Verma D, Talegaonkar S, Padhi S, Panda AK, Iqbal Z. *et al.* 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.
12. Yuan J, Guo L, Wang S, Liu D, Qin X, Zheng L. *et al.* Tian C, Han X, Chen R, Yin R. Preparation of self-assembled nanoparticles of ε-polylysine-sodium alginate: A sustained-release carrier for antigen delivery. Colloids and Surfaces B: Biointerfaces. 2018; 171:406-412.
13. AL-Quadeib BT, Radwan MA, Siller L, Horrocks B, Wright MC. Stealth Amphotericin B nanoparticles for oral drug delivery: *In vitro* optimization. Saudi Pharmaceutical Journal. 2015; 23(3):290-302.
14. Li Z, Lin X, Shen L, Hong Y, Feng Y. Composite particles based on particle engineering for direct compaction. International journal of pharmaceutics. 2017; 519(1-2):272-286.
15. Mahmoudi M, Bertrand N, Zope H, Farokhzad O. Emerging understanding of the protein corona at the nano-bio interfaces. Nano Today. 2016; 11(6):817-832.

16. Chan HF, Ma S, Leong KW. Can microfluidics address biomanufacturing challenges in drug/gene/cell therapies? Regenerative biomaterials. 2016; 3(2)87-98.
17. Hart DJ, Waldo GS. Library methods for structural biology of challenging proteins and their complexes. Current opinion in structural biology. 2013; 23(3):403-408.
18. Li Z, Lin X, Shen L, Hong Y, Feng Y. Composite particles based on particle engineering for direct compaction. International journal of pharmaceutics. 2017; 519(1-2):272-286.
19. Mahmoudi M, Bertrand N, Zope H, Farokhzad OC. Emerging understanding of the protein corona at the nano-bio interfaces. Nano Today. 2016; 11(6)817-832.
20. Kashi TSJ, Eskandarion S, Esfandyari-Manesh M, Marashi SMA, Samadi N, Fatemi SM. *Et al.* Atyabi F, Eshraghi S, Dinarvand R. Improved drug loading and antibacterial activity of minocycline-loaded PLGA nanoparticles prepared by solid/oil/water ion pairing method. International journal of nano medicine. 2012; 7:221.
21. Lim EK, Kim T, Paik S, Haam S, Huh YM, Lee K. *et al.* Nanomaterials for theranostics: recent advances and future challenges. Chemical reviews. 2014; 115(1):327-394.
22. Bouillot P, Ubrich N, Sommer F, Duc TM, Loeffler JP, Dellacherie E. *et al.* Protein encapsulation in biodegradable amphiphilic microspheres. International journal of pharmaceutics. 1999; 181(2):159-172.
23. Chan HF, Ma S, Leong KW. Can microfluidics address biomanufacturing challenges in drug/gene/cell therapies? Regenerative biomaterials. 2016; 3(2):87-98.
24. Meng Y, Wang S, Li C, Qian M, Zheng Y, Yan X. *et al.* Huang R. TKD peptide as a ligand targeting drug delivery systems to memHsp70-positive breast cancer. International journal of pharmaceutics. 2016; 498(1-2):40-48.