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Preparation and physicochemical characterization of novel anticancer ck-10 loaded nanoparticles to check biodegradation, compatibility and stability

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Abstract
The main objective of this project was to formulate novelamphiphilic PLGA nanoparticles having better physicochemical properties for the delivery of the novel peptide (CK-10) to the cancerous/tumour tissue. Double emulsion/Solvent evaporation [DE/SE] and a novel microfluidic techniques were used to formulate the nanoparticles. Size & images were characterized by laser obscuration time whereas *invitro* release were measured by modified Lowry assay. Stability was checked by high performance liquid chromatography and capillary zone electrophoresis. Evaluation of the compatibility, interaction and shelf life stability were confirmed by FTIR and differential scanning calorimeter. Water absorption and its associated changes in the physicochemical properties were measured by various color indicator and potentiometric titrations techniques. PLGA/Poloxamer & PLGA/βCyclodextrin nanoparticles produced by the novel microfluidic technique showed the highest in-vitro release with good size criteria and optimum biodegradation for the CK10 delivery to the cancer cells. Better physicochemical properties for the CK-10 loaded PLGA nanoparticles could be obtained by using the novel microfluidic technique to improve the RAN blocking by CK-10.

Keywords: CK-10, RAN, PLGA, Cancer

Introduction
Nanoparticulate formulations can be a step forward towards improved drug delivery [1-7]. The adjustment in sizes between microparticles (MP) and nanoparticles (NP) has marked effects. Size has a strong effect on the purpose of particles injected into the vasculature. Micro particles can simply make embolism to the vessels, which occludes blood flow, with or without parallel drug delivery. This drawback can be escaped by using nanoparticles that are too small to make embolism conditions and can circulate through the vasculature [2, 3, 4]. MP(s) can be transported to the phagocytic cells, whereas NP(s) are targeted to all kinds of cells. Formulation of polymeric NPs is more possible than MPs because the high shear vital for micro particles is needless for NPs. The larger surface area of NPs allows more payload encapsulation than MPs [1, 4]. In1999, the US FDA agreed a PLGA microsphere formulation, Nutropin Depot®, as a once-a-month substitute to daily injections of human growth hormone.
Blended polymer systems have been commonly studied owing to the outcome of water on the performance of biodegradable polymers and the critical part played by water-polymer interactions in biological procedures. In the existence of water, a polymer may display changes in mechanical, chemical, rheological, and transport properties [6, 7]. Depending on the chemical nature of the polymers in the blend, the incidence of water may result in definite changes, causing an intense influence on the performance of the polymer. For polymers that are biodegradable like PLGA, PCL, the effects of water may allow additional significance on the physicochemical properties of the whole formulations e.g. Glass transition temperature, stability, and polymer degradation that need to be investigated.

Materials and Methodology
All the chemicals were purchased from Sigma Aldrich UK. The novel CK-10 peptide was purchased from GL Biochem in China where it was made by custom synthesis/

Preparation of the nanoparticles by double mulsion/ solvent evaporation (de/se) technique.
1mg CK-10 or 1mg CK-10 and 5mg of the amphiphilic polymer were dissolved in 1ml of internal aqueous phase (2.5% m/v PVA) and mixed with 5 ml dichloromethane (DCM) containing 20 % m/v polymer, then emulsified at 10000 rpm (Silverson L5T, Silverson Machines, UK) for 2min. 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. The final emulsion n was stirred by magnetic agitation overnight under vacuum to evaporate the DCM. After the NP(s) had been formed, they were centrifuged (sigma centrifugation apparatus, Sigma, UK) at10000 rpm for 30 min at 4°C,which was enough to pellet the NP(s).The pellet was washed three times with distilled water and lyophilised at -85 ^{0}C& 0.012 mbar (Labconco freeze dryer, Free zone 4.5 Plus, UK) for 48 h [2, 3].

Preparation of The Nano particles By Micro fluidics Technique

The NPs of PLGA and PLGA blended polymers were prepared by a novel microfluidic technique using the nano Assembler ™ Bench top Instrument; Precision Nano Systems, Ltd., Vancouver, BC. 4 ml of the acetonitrile solution of PLGA (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 or the hydrophilic polymer were mixed in the microfluidic mixing chamber at a flow rate of (12 mL/min) by syringes indicated in the Nano Assemblr ™ software. The NP(s) were recovered by salting out using a 20 ml K2HPO4 (150mM) [1, 7].

Determination of Ck-10 *In Vitro* Release From The Various Nanoparticles Nano particles By Modified Lowry Protein Assay Method

A sample of peptide-loaded NP (5.0 mg) was suspended in 1.0 ml PBS (pH 7.4) solution and incubated at 37 0C with a shaking water bath. Samples were withdrawn at predetermined time intervals and replaced with fresh PBS, centrifuged for 5 min at 5000 rpm and the peptide concentration in the supernatant was determined by the microplate Lowry method. CK-10 absorption were measured by using of a minimum of three absorption determinations for each standard, blank and test samples [8].

Determination of nanoparticle size by laser obscuration time technique (lot).

Nanoparticles suspension was prepared by dispersing 5 mg of the nanoparticles in 1 to 2 ml double distilled ddH2O, then measured straightforward by a Beckman–Coulter laser obscuration time instruments with regard to Z-average diameter [9, 10].

Laser obscuration time technique (LOT) for the determination of nanoparticles morphology

LOT is useful to describe the morphology and size of the nano/micro particles [11]. Nanoparticles suspension was prepared by dispersing 5 mg of the nanoparticles in 1 to 2 ml double distilled ddH2O, then measured straightforward by a Beckman–Coulter laser obscuration time instruments.

Assessment of peptide

The stability of loaded peptide was determined after 14 days of *in vitro* release from the NP(s). The aqueous *invitro* release medium was analysed using HPLC–MS (Applied Bio systems/C18 reversed phase column, API4000 LC/MS/MS) and capillary zone electrophoresis (CZE) [12, 13]. The HPLC/MS included the used of the mobile phase, at a 1.0 ml/min flow rate, comprised a linear gradient of solvent B (0.1% TFA in acetonitrile) in solvent A (0.1% TFA in water) over a 30 min run time. Regarding (CZE), the aqueous *invitro* release medium was checked for the CK-10 stability by basic fused silica capillary zone electrophoresis (CZE). Constant voltage polarity setting (Positive-to-negative Running voltage: 10kV), current limit: 100µA at high pressure detector mode single wavelength: 200nm, running time (stop time):15 minutes & rise time: 1second were used for CZE operation.

Evaluation of the compatibility, interaction and shelf life stability

The compatibility and interaction between the peptide and the NP(s) were checked by the differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR) [12-15]. DSC scans were performed on a Mettler DSC 823 (Mettler Toledo, GmbH, Switzerland) and Infrared (IR) spectra of freeze-dried nanoparticles were obtained with a Perkin-Elmer 1600 spectrophotometer (GMI Inc., Ramsey, Minnesota). Shelf life stability of the NP(s) was checked at ambient room temperature, 5 ^{0}C and accelerated condition at 35 ^{0}C using FTIR.

Determination of water uptake and water-Soluble Acids (lactic & glycolic) released during Polymer Degradation

Approximately 10 mg of dried polymer was incubated in 2 ml distilled water for one week at room temperature. The supernatant was separated from the NP(s) by centrifugation at 5000 rpm under 4 ^{0}C.removed. The NP(s) pellet were vacuum dried until constant weight. 1mL of anhydrous methanol was added to the NP(s) pellet and the samples were occasionally shaken for 1 hour. A small volume (UL) of methanol was withdrawn and injected into the Karl Fischer titration vessel [15, 16]. Water content was calculated as follows:

Water content (%) = 100 × (M_{water} −M_{blank})/$M_{initial}$ Where M_{water} is the mass of water in the sample, M_{blank} is the mass of water in methanol, and $M_{initial}$ is the initial mass of the polymeric NP(s). Total water-soluble acid content inside PLGA during polymer degradation was calculated according to the following steps. Approximately 10 mg of dried polymer was incubated in 2 ml distilled water for one week at room temperature. The supernatant was separated from the NP(s) by centrifugation at 5000 rpm under 4 ^{0}C and was measured by the pH meter and was then dissolved in 20–40mL of a mixture of acetone/tetra hydro furan (1:1) and immediately titrated with 0.01N alchoholic potassium hydroxide (KOH) by Metro hm 794 Basic Titrino to a stable pink end point using phenolphthalein as an indicator.

Results and discussion

The nanoparticles were prepared by the technique DE/SE and a modified microfluidic technique. The ratios of PLGA amounts, hydrophilic and amphiphilic polymers amounts and theoretical CK 10 loadings used for preparation of the various nanoparticles are shown in table 1.

Table 1: The ratios of PLGA & amphiphilic PLGA/blend(s) amounts and CK-10 loading using amphiphilic polymers(PEG, Poloxamer & PVP) and hydrophilic polymers(β-cyclodextrin, HPMA, PA & PEI)

Polymersused	TheoreticalCK-10loading
PLGA(30000-60000)-DE/SE(F1)	1%
PLGA-N(F2)	1%
PLGA/Polyethyleneglycol4000(PEG) DE/SE5%(F3)	1%

PLGA/PEG-N5%(F4)	1%
PLGA/Poloxamer(Pluronic188)5%-DE/SE(F5)	1%
PLGA/Poloxomer5%-N(F6)	1%
PLGA/Polyvinylpyrolidone(10000)5%-DE/SE(F7)	1%
PLGA/PVP5%-DE/SE(F8)	1%
PLGA/β-cyclodextrin DE/SE5%(G3)	1%
PLGA/β-cyclodextrinN5%(G4)	1%
PLGA/Poly(N-(2-hydroxypropyl)methacrylamide) (HPMA),averageMn30,000-50,000,5%-DE/SE(G5)	1%
PLGA/HPMA5%-N(G6)	1%
PLGA/Polyethylenimine, linearaverage Mn5000,(PEI)5%-DE/SE(G7)	1%
PLGA/PEI5%-N(G8)	1%
PLGA/Polyacrylicacid,averageMw~100,000(PA),5%-DE/SE(G9)	1%
PLGA/Polyacrylicacid,averageMw~100,000(PA),5%-N(G10)	1%

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

The NP(s) were characterized by the same methods under the same conditions. Laser anemometry technique was used for zeta potential whereas LOT and dynamic light scattering (DLS) techniques were used for size measurement. The modified lowry assay was used for the *in vitro* peptide release and, SEM, LOT & TEM were used for morphological identification & uniformity evaluation of the nanoparticles (table 2).

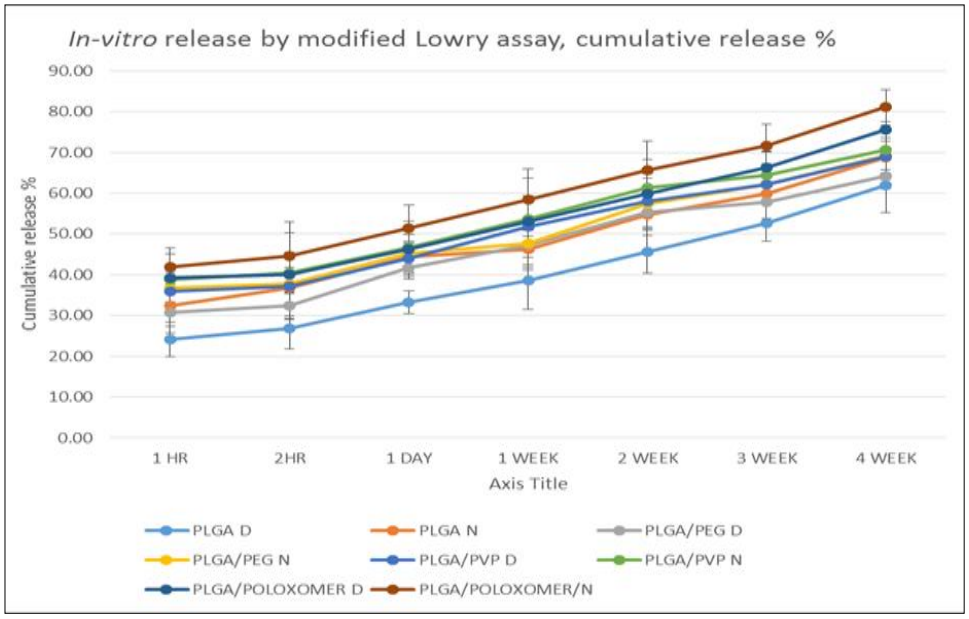
Table 2: Physicochemical Characterization of the Various PLGA NP(s)

CK-10 loaded polymer	F1	F2	F3	F4	F5	F6	F7	F8
Z-average, nm ± s.d, by LOT, n=3	264.33 ± 5.51	249.07 ± 13.15	242.83 ± 2.93	223.57 ± 9.21	219.10 ± 8.80	211.83 ± 4.15	245.27 ± 11.72	230.10 ± 15.48
Water content % by Karl Fischer titration ± s.d., n=3	2.34 ± 0.24	2.71 ± 0.13	3.29 ± 0.38	3.53 ± 0.51	3.59 ± 0.16	3.93 ± 0.47	3.42 ± 0.11	3.63 ± 0.47
CK-10 loaded polymer	G3	G4	G5	G6	G7	G8	G9	G10
Z-average, nm ± s.d, by LOT, n=3	265.83 ± 2.93	253.57 ± 9.21	270.10 ± 8.80	257.83 ± 4.15	273.27 ± 11.72	265.27 ± 11.72	271.27 ± 11.72	265.10 ± 15.48
Water content % by Karl Fischer titration ± s.d., n=3	3.77 ± 0.38	4.13 ± 0.51	3.29 ± 0.16	3.53 ± 0.47	3.44 ± 0.11	3.72 ± 0.11	3.52 ± 0.11	3.79 ± 0.47

In vitro release

Peptides like CK-10 have a hydrophilic nature and can be highly attached to hydrophilic moieties of the hydrophilic or amphiphilic polymers like PEG, PVP and poloxamers [17, 22]. Additionally, amphiphilic polymers can also act as surfactants by accumulating at the internal interface of the particle to help the peptide spread throughout the innermost phase.

All the microfluidic nanoparticles had higher protein release rates than DE/SE nanoparticles. Addition of the amphiphilic polymers enhanced the release rates (fig.1). When the *in vitro* release study was conducted using the PBS,it was found that the cumulative amount of the *in vitro* release in 4 weeks was 81.11&75.56% in case of F5&F6 respectively which were the highest values (fig.1). Nevertheless all the amphiphilic blends could reach higher release rates than the PLGA alone.



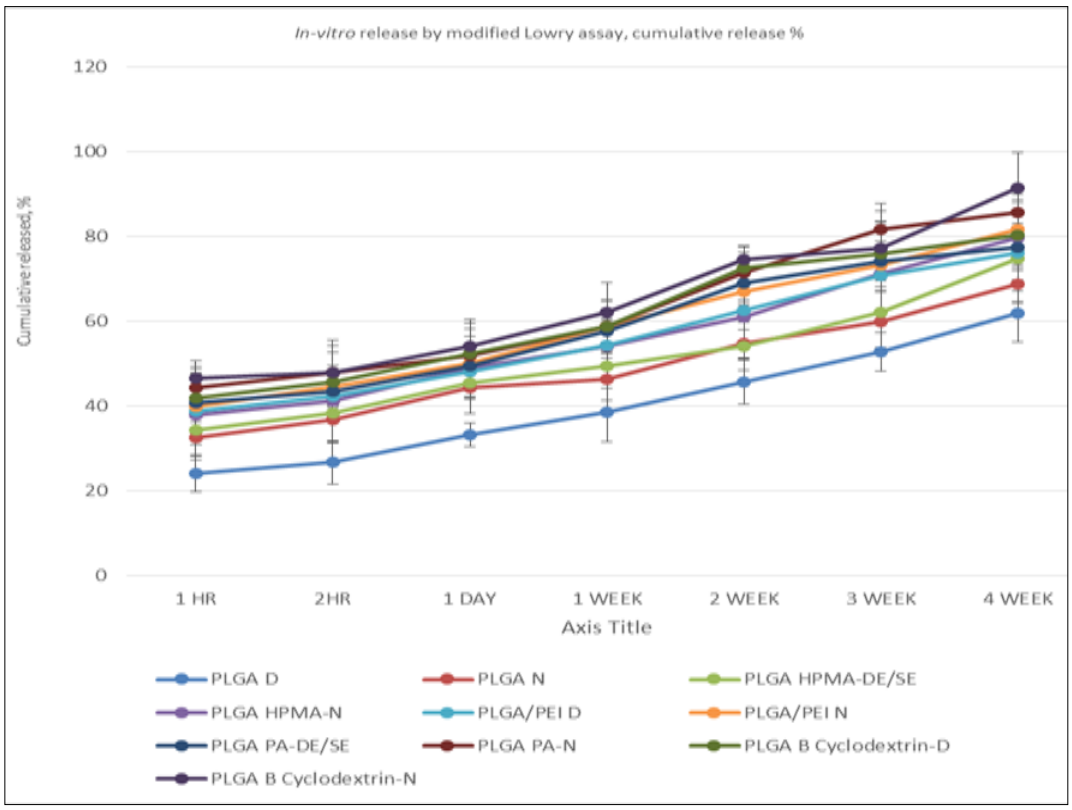


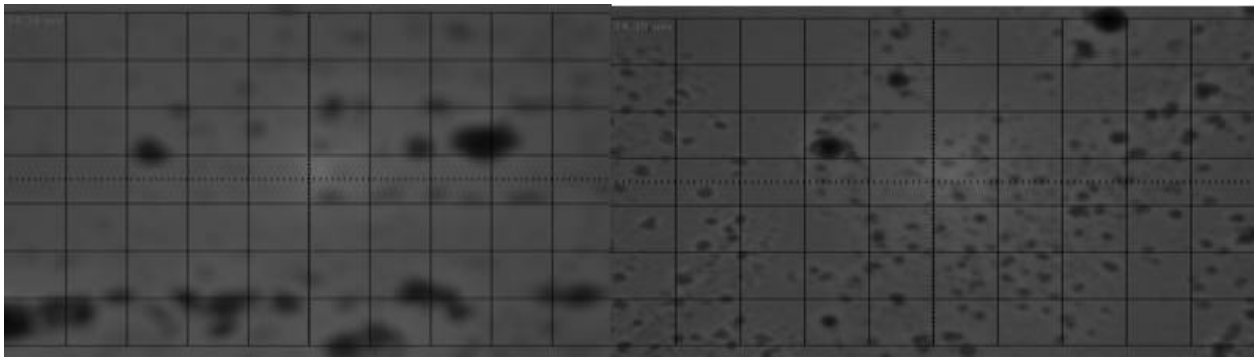
Fig 1: *In vitro* release of CK 10 NP(s).

Totally, the microfluidic nanoparticles had higher peptide release proportions than DE/SE nanoparticles. Adding of the hydrophilic polymers enhanced the release rates. When the *in vitro* release study was piloted using the PBS, it was observed that the collective amount of the *in-vitro* release in 4 weeks was 91.38 & 80.27 % in case of G9 & G10 correspondingly which were the maximum values(Fig.1). Higher loading efficiency for the drugs might have been achieved, was accredited to the reduced drug loss due to fewer steps used in microfluidic-assisted production compared with consecutive bulk mixing of multiple phases in emulsification procedures [17, 18, 19]. Moreover, the droplets and three-dimensional micro channel geometries of the microfluidics device like the nano assembler result in composite folding of fluid flows, which can entirely mix two or more streams in milliseconds [20, 21, 22].

Size

The use of the nano assemlbr declined the size ranges of all the nanoparticles than the DE/SE technique by the laser obscuration technique (Fig.2 & table 1). PLGA/Poloxamer had the lowest size ranges (219.10 for DE/SE and 211.83 for the microfluidic nanoassemblr. The use of the nanoassemblr reduced the size series of all the nanoparticles compared to the DE/SE (Fig.2 & table 1).

Though, the hydrophilic polymers blends had larger sizes than the PLGA for both of the used techniques (table 1).The nanoassembler microfluidic technique succeeded in decreasing the size and narrowing the PDI of the PLGA and PLGA blends (table 1). The amphiphilic polymers exhibited narrower PDI(s) than the PLGA for both of the formulation techniques (fig.2 & table 1).



LOT images of PLGA/β-cyclodextrin NP(s) prepared by DE/SE LOT images of PLGA/β-cyclodextrin NP(s) prepared by Micro fluid Icsnanoa Asembler

Fig 2: LOT images of PLGA/Poloxamer NP(s)

Small sized nanoparticles formulated in batch or bulk by conventional methods suffer from the high polydispersity index (PDI). In contrast, the microfluidic tactics empowers the manufacture of both amorphous and crystalline mono dispersed nanoparticles [23, 25]. At the high speed mixing and flow considered for the microfluidic devices, nanoparticles acquire uniform morphology with smaller size and narrower PDI. This is because at high speed flow, thickness of liquids is reduced, which in turn adjusts local super saturation and finer nanoparticles are obtained compared to the conventional techniques.

Nanoparticle production takes place in the micro droplets to increase the mixing efficiency in the microfluidic channels, and further shrinks the particle size distributions. It is evidently shown that this 3D technology expressively progresses the sandwiching of solvent/ polymer phase, as a result it is beneficial for the excellent mixing of the two phases for nanoprecipitation and marks in significantly decreasing the size of the nanoparticles. Limited diffusion within the microdroplets and rapid mixing as long as the droplets move in the micro channels smooth charge neutralization between the molecules. In the emulsification methods, these parameters are hard to control, leading to rough mixing, limited temperature fluctuations and unrestrained reaction times. In contrast, microfluidic devices permit for control over the mixing time by changing solvent flow rates or channel geometry. The reduction of size & PDI ranges for all the blends, compared to the DE/SE technique is mainly reliant on the hydrodynamic flow rate of the nano assemblr.

Water absorption by the various PLGA NP(s) and its associated effects on pH & acid content.

Water entrance resulted to series of noteworthy events like the hydration & degradation of the PLGA and finally the solubilization PLGA degradation products (table.3).

Table 3: Effect of water absorption on the PH of the degraded acids of PLGA By acid base titration and PH meter

Polymericnanoparticles	Conc of the degraded acids(lactic & glycolic) of PLGA By acid base titration(µmol/mg)±SD,N=3	EMF of the degradation products of PLGA By Acid base titration(mV)±SD,N=3	pH of the degraded acid of PLGA By acid base titration(water content, %)±SD,N=3
PLGA-DE/SE	0.26±0.04	35.32±3.36	6.83± 0.11
PLGA-N	0.3±0.072	37.13±3.32	6.71±0.07
PLGA chitosan-DE/SE	0.32±0.03	40.67±2.52	6.60±0.58
PLGA/β-cyclodextrin-D	0.41± 0.03	48.05±3.51	6.59±0.52
PLGAPEG-DE/SE	0.37± 0.04	46± 5.1	6.49±0.47
PLGAPVP-DE/SE	0.39± 0.072	49.23±7.01	6.48±0.47
PLGAHPMA-DE/SE	0.43±0.045	52.52± 3.8	6.38±0.26
PLGAβ-cyclodextrin-N	0.51±0.056	59.54±3.27	6.29±0.15
PLGA poloxomer-DE/SE	0.44±0.097	54.49±6.77	6.32±0.51
PLGAPA-DE/SE	0.42±0.04	53.92±7.4	6.32±0.18
PLGAPEG-N	0.45±0.09	55.06± 5.059	6.26± 0.20
PLGAPEI-DE/SE	0.34±0.05	42.67±4.7	6.47± 0.12
PLGAHPMA-N	0.36±0.052	43.35±2.26	6.39±0.13
PLGApoloxomer-N	0.63±0.04	79.37±0.91	6.08± 0.20
PLGAPEIN	0.37± 0.044	44.64±8.81	6.41± 0.08
PLGAPVP-N	0.38± 0.1	48.03±1.87	6.38±0.08
PLGAPA-N	0.45±0.07	56.18±5.15	6.28± 0.23
Polymericnanoparticles	ConcofthedegradedacidofPLGABypH meter (µmol/mg)±SD,N=3	EMFofthedegradationproductsofPLGABy pHmeter(mV)±SD,N=3	pHofthedegradedacidofPLGAByPHmeter (watercontent,%)
PLGA-DE/SE	0.29±0.14	36.66±3.062564	6.69± 0.39
PLGA-N	0.36± 0.17	38.79±2.4	6.41±0.31
PLGA chitosan-DE/SE	0.39±0.13	42.67±1.78	6.16±0.87
PLGAβ-cyclodextrinD	0.45± 0.13	45.05±5.37	6.07±0.71
PLGAPEG-DE/SE	0.41± 0.14	42.67±2.14	5.96±0.84
PLGAPVP-DE/SE	0.49±0.17	50.57±3.05	5.81±0.43
PLGAHPMA-DE/SE	0.51±0.145	53.85±4.79	5.77±0.42
PLGAβ cyclodextrin-N	0.52±0.1	57.83±10.55	5.62±0.50
PLGApoloxomerDE/SE	0.54±0.197	60.87±8	5.46±0.51
PLGAPA-DE/SE	0.43± 0.14	46.54±5.26	5.82±0.04
PLGAPEG-N	0.47±0.19	48.39±8.82	5.76± 0.38
PLGAPEI-DE/SE	0.37±0.15	41.23±8.76	6.1± 0.46
PLGAHPMA-N	0.54± 0.151	56.68±3.57	5.52±0.13
PLGA poloxomer-N	0.66±0.14	72.71±6.28	5.15± 0.38
PLGAPEIN	0.33± 0.14	47.98±12.7	6.34± 0.50
PLGAPVP-N	0.54±0.2	59.36±6.98	5.4±0.53
PLGAPA-N	0.48±0.27	49.39±8.57	5.64±0.40

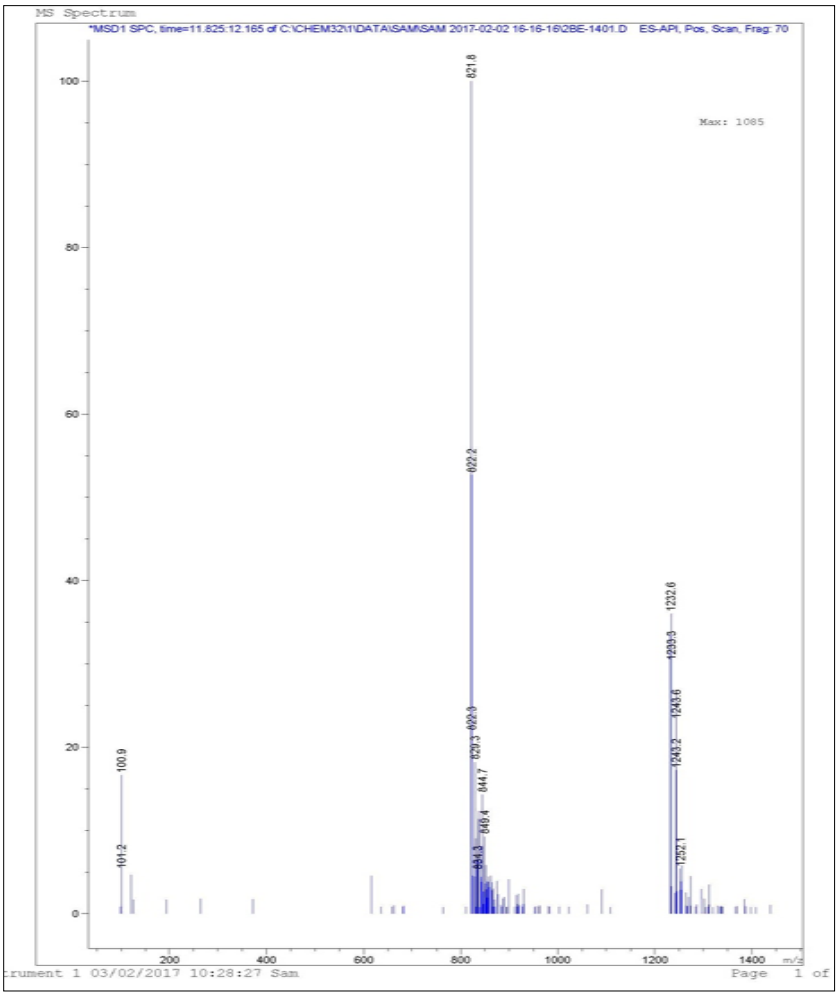
Table. 3. Illuminated the moisture uptake by the polymer throughout the early stages of hydration which were central to

the considerate of the biodegradation procedure. Interestedly, the amount of water uptake by the amphiphilic NP(s) incubated in distilled water was superior to that detected for the PLGA alone. The bigger capacity of liquid bulk water penetrating the amphiphilic NP(s) are connected by higher PLGA degradation and consequently by solubilization of its degradable products, which could be straightforwardly measured by the acid base and potentiometric titration parameters (total water soluble acid content, PH of the water-soluble acid content & EMF of the water-soluble acid content). In the same way to the classifying of the loading efficiency and release, β-cyclodextrin could demonstrate the highest water absorption allied by the highest PLGA degradation and solubilisation of its products. Water uptake by the β-cyclodextrin blends (G3, G4) (3.77, 4.13) resulted to whole water soluble acid content (0.41, 0.51) by having PH(s) (6.59, 6.29) and EMF(S) (48.05, 59.54). Similarly to the loading and release values, all the NP(s) expressed by the microfluidic technique had advanced release rates matched to those formed by the DE/SE procedure. PLGA polymers are thermoplastic materials that adsorb water without dissolving in aqueous solutions [18-30]. Hence, NP(s) water uptake measurement throughout the initial stages of hydration is critical to understand the biodegradation process. Blending of the PLGA with amphiphilic polymers enhanced the water uptake. The smaller sizes achieved by the microfluidic technique resulted into higher surface area which could improve the water uptake for the microfluidic NP(s) by higher. Consequently, the sequential process of water soluble acids formation was higher associated by lower PH values to expressively prove the enriched water uptake. This phenomena could improve the release of the peptide within the cancer cells.

PLGA polymers adsorb water without solubilization in Aqueous solutions [18-30]. Hereafter, NP(s) water uptake quantification during the early stages of hydration is serious to recognize the biodegradation progression. Combination of the PLGA with hydrophilic polymers improved the water uptake. The reduced sizes attained by the microfluidic technique ensued into higher surface area which could advance the water uptake for the microfluidic NP(s). Subsequently, the chronological process of forming water soluble acids was higher connected by lesser pH values to vividly verify the enhanced water uptake. These occurrences could increase the release of the peptide inside the cancer cells.

Stability, compatibility of the encapsulated peptide and Shelf life stability of the NP(s)

Fig. (3 & 4) show similar peaks in HPLS/MS CHARTS (1234 m/z) & similar retention time in CZE (4.5) in the PLGA & blended PLGA nanoparticles.



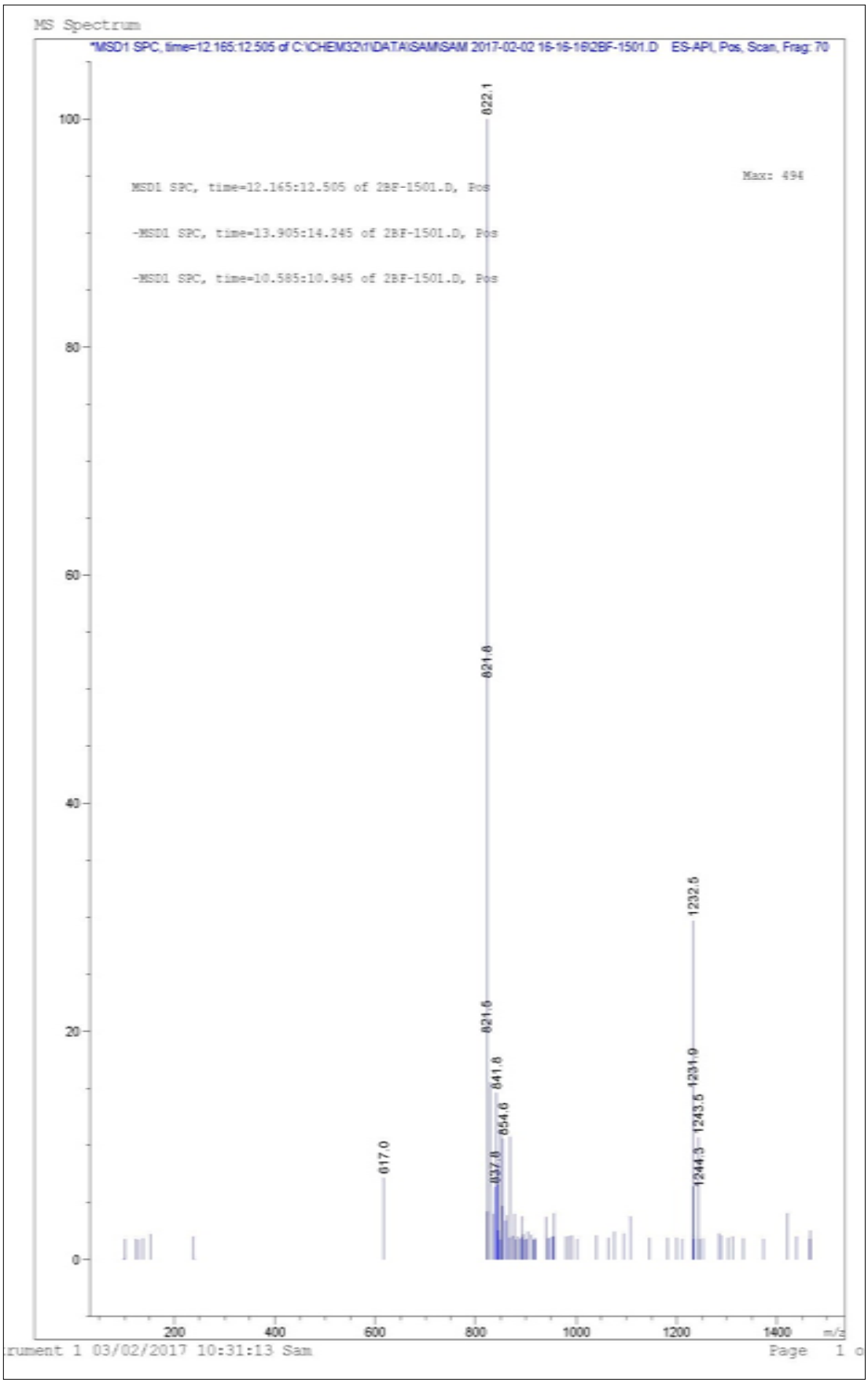
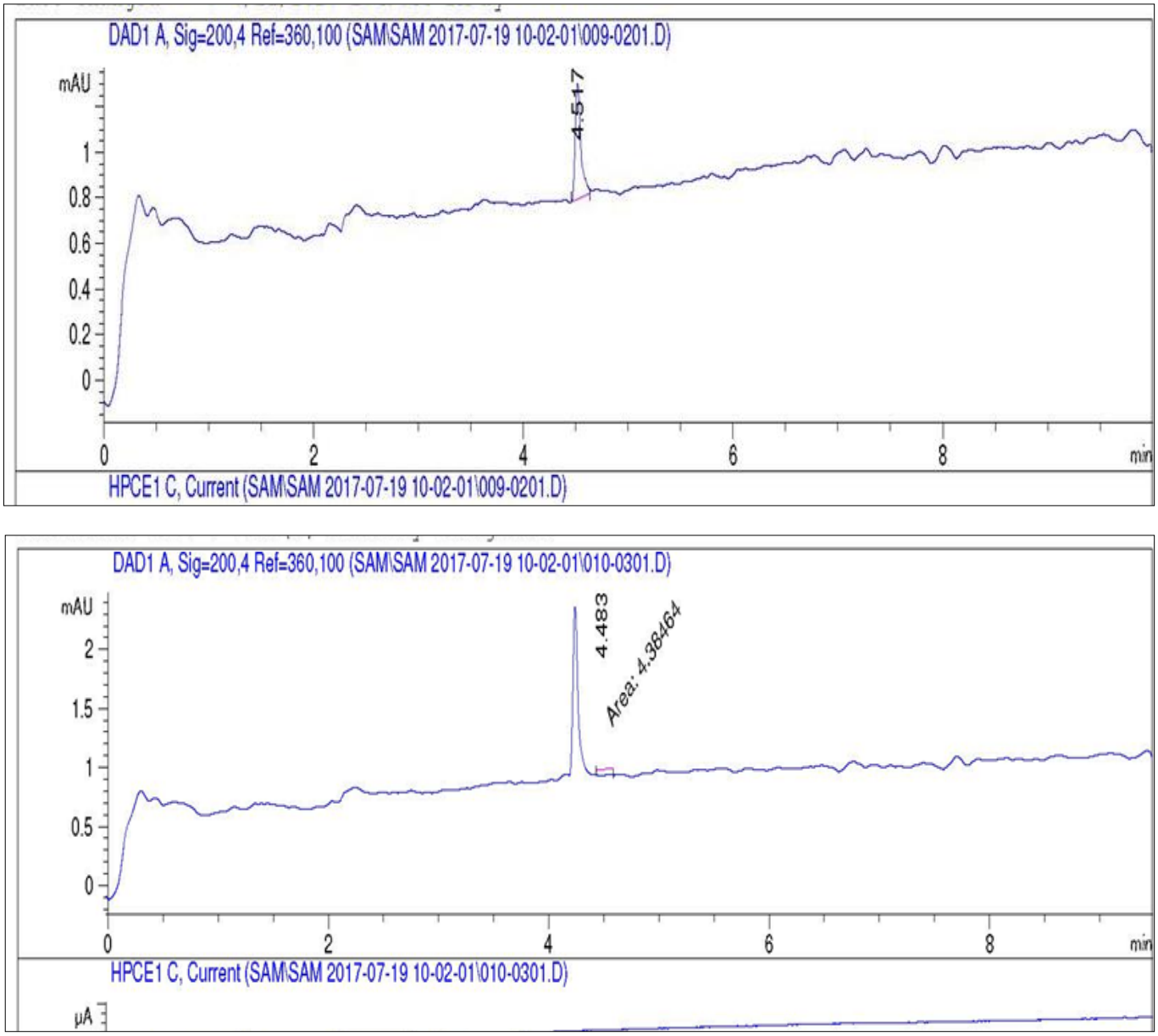


Fig 3: HPLC/Ms chart of reference CK-10 peptide HPLC/Ms chart of CK-10 released from PLGA/B β-cyclodextrind NP(s) prepared by DE/SE the *in vitro* aqueous release medium Fig.4. HPLC/Ms Charts showing CK-10 stability.

CK-10 stability is similar in the various PLGA nanoparticles regardless the Mwt& type of the amphiphilic polymer. Additionally, it is clear that the amphiphilic polymers don't provide more CK-10 stability than PLGA. The similar peaks between the released peptide and the reference in figures 3, 4 [23, 24, 25] obtained by CZE and HPLC-MS, could approve the stability of the loaded CK-10. The stability parameter is very important for the efficacy of the drug and hence, it was furthermore established by the parallel mass spectrum using the HPLC-MS (1234 m/z) and the parallel retention time by the CZE (4.5).



CZEchartofreferenceCK-10 peptide. CZE chart of CK-10 released from PLGA/poloxamer NP(s).N the *in vitro* aqueous release medium

Fig 4: CZE charts showing retention time of CK-10.

The amphiphilic polymers in the NP(s) are not only oriented towards the external aqueous phase but also towards the inner aqueous phase. Thus, the CK-10 reservoir can be surrounded by an amphiphilic barrier which protects it during the NP formation. All the amphiphilic nanoparticles don't provide more CK-10 stability than the PLGA nanoparticles because the CK-10 protection by the hydrophobic interactions and physical cross links of PLGA is sufficient without the amphiphilic barrier.

This confirms that the CK-10 stability weren't affected by the physicochemical properties of the amphiphilic polymers or the techniques used. This could clarify the similarity of the CK-10 peaks in HPLC–MS & CZE. The hydrophilic polymers in the NP(s) can be oriented towards the external and internal aqueous phases during the NP formation [24, 30]. Thus, the CK-10 reservoir can be fenced by a hydrophilic barrier which guards it during the NP formation. All the amphiphilic nanoparticles don't afford more CK-10 stability than the PLGA nanoparticles due to the sufficiency of the hydrophobic

interactions and physical cross linking of PLGA to protect the CK-10.This confirms that the CK-10 stability and shelf life stability at various conditions weren't affected by the physicochemical assets of the hydrophilic polymers or the procedures used. This could clarify the similarity of the CK10 peaks in HPLC–MS & CZE as well as the parallel of the charts in the FTIR (fig.3, 4, 6).

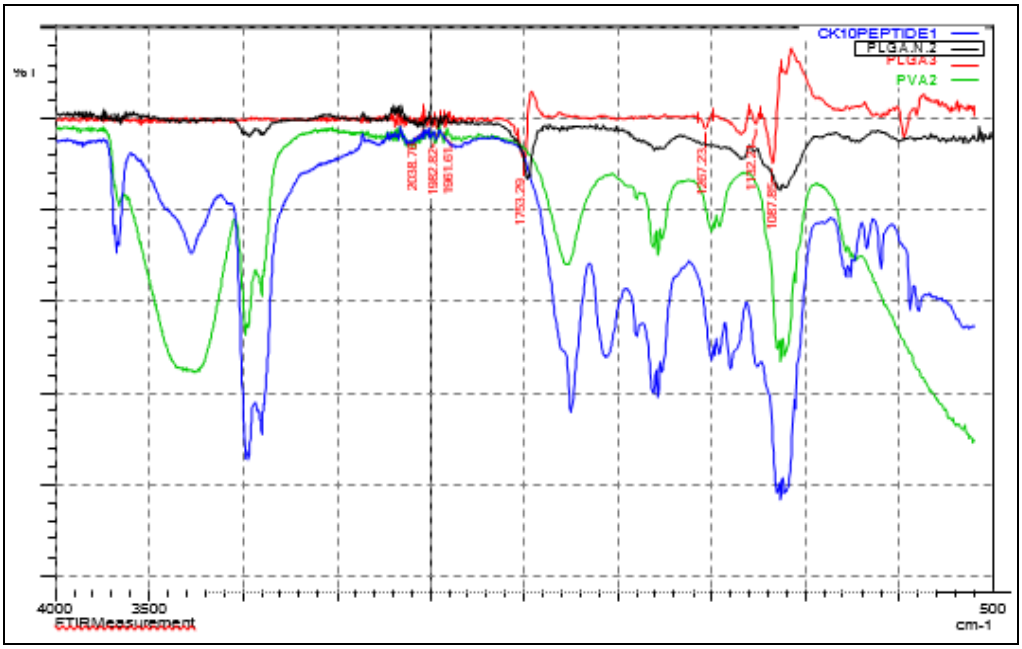


Fig 5: FTIR charts showing the successful loading of the hydrophilic polymer(s) and CK-10 peptide within the PLGA

The major peaks assigned to the individual ingredients confirmed the presence of different groups. The peaks at 3000-3500 cm^{-1}due to presence of O-H(stretching) & N-H(stretching),1753cm^{-1}duetopresence(C=O,stretching) of the PLGA, 1640cm^{1} due to presence of NH-C=O,C=O,(stretching, amide) of the CK-10 peptide and 1500cm^{-1} due to presence of NH (bending). It was confirmed that final NP(s) totally had the same peak of 1750cm^{-1}, indicating that there is no shifting for the PLGA functional group carbonyl as well as loss of functional peaks of the other ingredients confirming the successful compatibility and encapsulation of the individual ingredients within the final NP(s) (fig.5). Shelf life of the various NP(s) at different conditions (RT, 5 ^{0}c, 35 ^{0}c) would not affect the stability of the NP(s) as the spectrum of NP(s) were constant during the various storage environments (Fig.6).

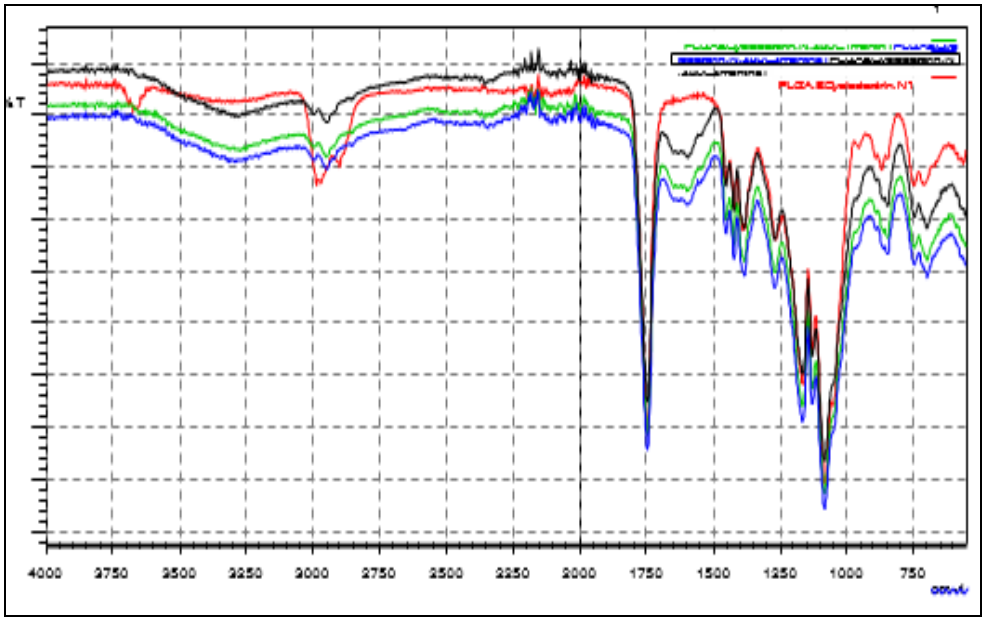


Fig 6: FTIR charts showing the shelf life stability of the PLGA/β-cyclo dextrin NP(s) at ambient room temperature, 50C and accelerated conditions 350C.

Polyesters have high compatibility with various hydrophilic and amphiphilic molecules [23, 30]. This explained the disappearance of all the peaks of the peptide and hydrophilic polymers if the final FTIR charts of the NP(s) to confirm the successful loading of the hydrophilic polymers and the CK-10 within the PLGA.The physicochemical properties may be modified by copolymerization or blending or grafting with other polymers. However, the melting point of PLGA were not affected by the hydrophilic or the amphiphilic polymers and the CK-10 due to their lower ratios in the NP(s).The physicochemical properties may be modified by copolymerization or blending or grafting with other polymers. However, the melting point of whole CK 10 NP(s) is 51 ^{0}C which is similar to the pure PLGA polymer (fig.7) for all the formulations in both of the used techniques.

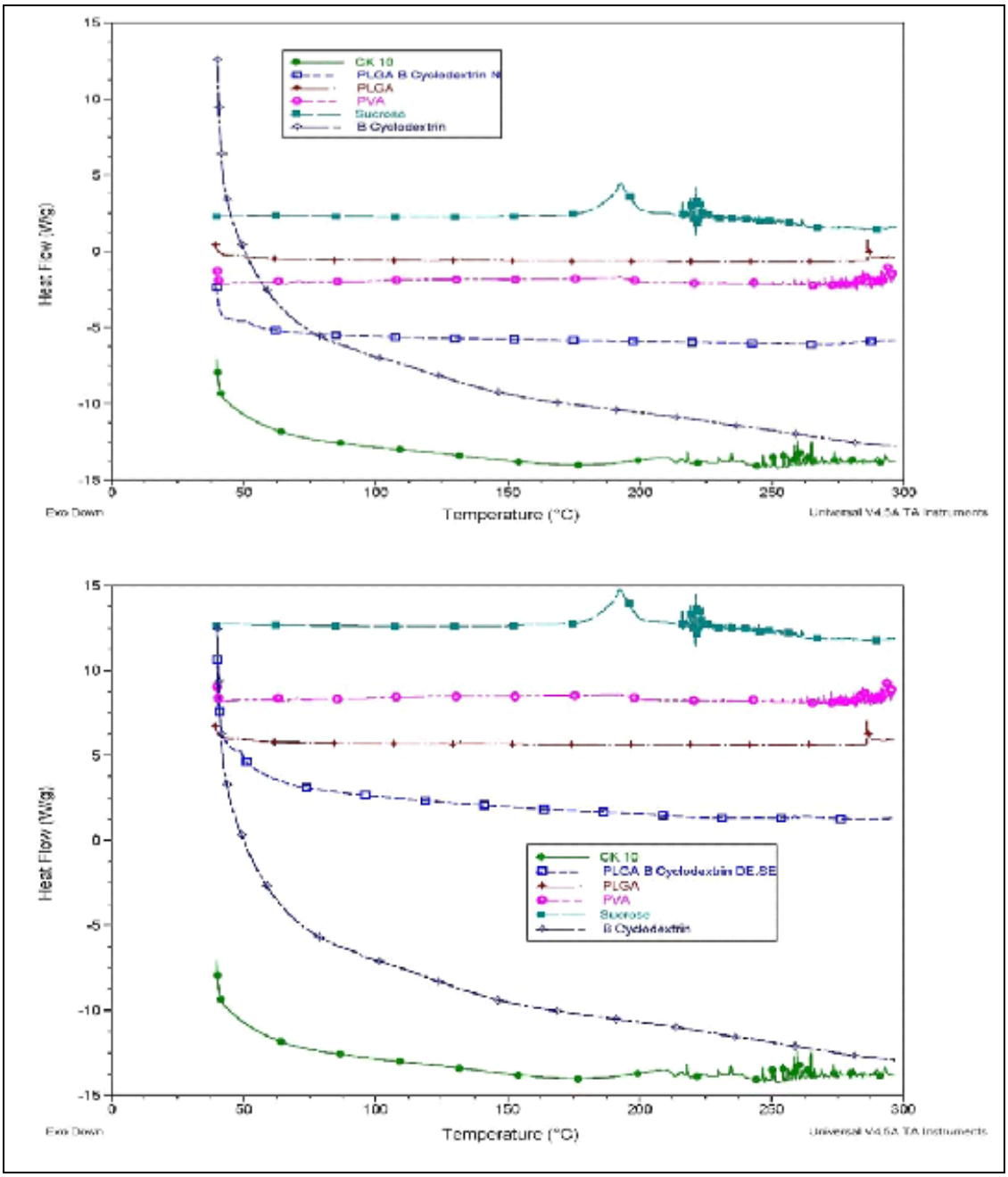


Fig 7: DSC charts showing the interaction of the hydrophilic polymer(s) and CK-10 peptide with the PLGA

Conclusion

Blending of amphiphilic polymers with PLGA could improve the physicochemical properties than PLGA nanoparticles, especially by using the novel microfluidic technique which could overcome several difficulties of the emulsification techniques like the DE/SE e.g. higher *in vitro* release rates, smaller sizes and higher uniformity. It could also accelerate the polymer degradation to improve the biodegradation of the CK 10 NP(s) within the cancer cells to release the anticancer peptide. PLGA/poloxamer and PLGA/β-cyclo dextrin blends

have the highly optimized physicochemical properties, especially by the novel microfluidic technique e.g. size & PDI, CK 10 loading efficiency, *in vitro* CK 10 release, and biodegradation. Therefore, PLGA/Polo xamer and PLGA/β-cyclodextrin nanoparticles would be selected for the selecting targeting design against the cancer cells. There were no differences regarding the stability of the encapsulated peptide and the shelf life stability of the novel CK 10 NP(s). The CK 10 NP(s) components are compatible in both of the used techniques showing successful incorporation of the CK 10 and the hydrophilic or amphiphilic polymers without affecting the melting point of the PLGA.

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