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# Using PVA and TPGS as combined emulsifier in nanoprecipitation method improves characteristics and anticancer activity of ibuprofen loaded PLGA nanoparticles

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In the preparation of nanoparticles (NPs) by the nanoprecipitation method, emulsifiers play a key role for NPs' characteristics. The present study aimed to investigate the combined emulsifier effect on ibuprofen loaded poly(-lactic-co-glycolic acid) (PLGA) NPs' characteristics and anticancer activity. Ibuprofen loaded PLGA NPs were prepared by nanoprecipitation using different concentrations of PVA (poly(vinyl alcohol)) or PVA–TPGS (d-α-tocopherol polyethylene glycol 1000 succinate) combination as emulsifier. It was found that encapsulation efficiencies of NPs varied between 17.9 and 41.9 % and the highest encapsulation efficiency was obtained with 0.5% PVA + 0.1% TPGS (coded as PLGA PVA/TPGS NPs). PLGA PVA/TPGS NPs were characterized and compared with PLGA PVA NPs, which was obtained by 0.5% PVA alone. Polydispersity index of PLGA PVA/TPGS and PLGA PVA NPs were found to be 0.08 and 0.15, respectively. Incorporation of TPGS with PVA slightly decreased the initial ibuprofen release. Transmission electron microscopy analyses demonstrated a nearly uniform particle size distribution and spherical particle shape of the PLGA PVA/TPGS NPs. Additionally, PLGA PVA/TPGS NPs were significantly more cytotoxic than PLGA PVA NPs on the MCF-7 (human breast adenocarcinoma cells) and Caco-2 (human epithelial colorectal adenocarcinoma) cells ($p<0.05$). Also PLGA PVA/TPGS NPs were not cytotoxic on normal cells (L929, mouse healthy fibroblast cells) ($p>0.05$). In conclusion, these results indicated that using a combination of TPGS and PVA as an emulsifier in nanoprecipitation could be a promising approach for preparing ibuprofen loaded PLGA NPs because of their improved characteristics and anticancer activity.

## 1. Introduction

Nanotechnology offers some advantages such as improved drug release, intracellular drug delivery and tumor accumulation by active and passive targeting properties (Hillaireau and Couvreur 2009; Wicki et al. 2015; Kamaly et al. 2016). Poly(lactic-co-glycolic acid) (PLGA) is the most frequently used polymer for preparing nanoparticles (NPs) because of its biodegradable and biocompatible nature (Dinarvand et al. 2011; Danhier et al. 2012). Also PLGA was approved by the United States Food and Drug Administration and The European Medicines Agency for medical use (Danhier et al. 2012). In recent years, water-insoluble drugs have been encapsulated in PLGA NPs (Sah and Sah 2015). Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) with poor water solubility (Harris 2015). Some data indicated that intake of NSAID's decreases cancer risk (Langman et al. 2000; Xu 2002; Baron 2003).

To prepare PLGA NPs, many methods were developed and nanoprecipitation is most commonly used (Schubert et al. 2011). Emulsifiers play a key role in the nanoprecipitation method, since characteristics of NPs such as average particle size, shape, particle aggregation, encapsulation efficiency (EE) could be affected by type and concentration of the emulsifier (Saadati and Dadashzadeh 2014; Sah and Sah 2015). Polyvinyl alcohol (PVA) and D-alpha tocopherol polyethylene glycol 1000 succinate (TPGS) are the most commonly used emulsifiers to prepare PLGA NPs by nanoprecipitation. Relatively uniform, small and dispersible NPs could be obtained with PVA, and for TPGS, an intrinsic anticancer activity was demonstrated (Sahoo et al. 2002; Feng et al. 2007; Duhem et al. 2014; Saadati and Dadashzadeh 2014; Sun et al. 2014).

The main objective of this study was to evaluate emulsifier effect on ibuprofen loaded PLGA NPs' characteristics and obtain an improved formulation against cancer. For this aim, ibuprofen loaded PLGA NPs were prepared by nanoprecipitation using different concentrations of PVA and PVA-TPGS in combination as emulsifier. NPs' formulations were selected by the encapsulation efficiency results. Finally, these formulations were fully characterized and anticancer activity of these formulations was evaluated on MCF-7 (human breast adenocarcinoma cells) (Yerlikaya et al. 2013) and Caco-2 cells (human epithelial colorectal adenocarcinoma cells) (Martins et al. 2015). Additionally, the cytotoxicity of formulations was evaluated with L929 cells (mouse healthy fibroblast cells) (Varan et al. 2016).

## 2. Investigations, results and discussion

The nanoprecipitation method provides many advantages for preparing PLGA NPs. Importantly, high energy/high pressure mixing devices are not needed for this method. Additionally, smaller NPs could be obtained by nanoprecipitation method compared to other methods (Sah and Sah 2015). Briefly, a polymer is dissolved in the water miscible organic solvent and this solution is diluted with water (antisolvent). Then, solvent breaks up and disperses as drops in the antisolvent and the NPs form via inter-

facial turbulence or the Gibbs–Marangoni effect. Until nano sized droplets form, these drops break down to smaller droplets. The solvent flows away from regions of low surface tension and the polymer precipitates (Mora-Huertas et al. 2011).

The low solubility of ibuprofen becomes a problem in the penetration through biological barriers, thus ibuprofen loaded NPs were studied in order to overcome this problem (Bonelli et al. 2012; Catarina Pinto Reis 2013; Vij et al. 2016). In this study, ibuprofen loaded PLGA NPs were prepared by nanoprecipitation to provide an easily producible and improved delivery system for ibuprofen.

Emulsifiers play a key role in the nanoprecipitation method and EE is one of the most important parameter for production of nanoparticles. In this method, diffusion and solubilization of drugs could be change by altering emulsifier type and amount. So, EE could be optimized by determining optimal concentration and type of emulsifiers (Saadati and Dadashzadeh 2014). In this study, EE values varied between 17.9 and 41.9 % (Table). The highest EE was obtained by using 0.5% PVA + 0.1% TPGS as 41.9 %.

**Table: Emulsifier effects on encapsulation efficiency of ibuprofen loaded PLGA NPs**

| Aqueous phase emulsifier concentration | Encapsulation efficiency (%) |
|---|---|
| **0.5 % PVA** | 21.9 ± 2.1 |
| **1 % PVA** | 26.8 ± 3.4 |
| **1.5 % PVA** | 23.6 ± 5.2 |
| **3 % PVA** | 15.4 ± 1.9 |
| **0.5 % PVA + 0.02 % TPGS** | 20.2 ± 3.5 |
| **0.5 % PVA + 0.1 % TPGS** | 41.9 ± 4.1 |
| **1 % PVA + 0.02 % TPGS** | 24.6 ± 3.7 |
| **1 % PVA + 0.1 % TPGS** | 17.9 ± 4.5 |

For further comparison between PVA alone and PVA in combination with TPGS, NPs were prepared using 0.5% PVA (PLGA PVA NPs) and 0.5% PVA + 0.1% TPGS (PLGA TPGS/PVA NPs) as emulsifier. This comparison showed that the addition of TPGS increased the EE and decreased the PDI and burst release of NPs (Table and Figs. 1-2). PDI of PLGA PVA/TPGS and PLGA PVA NPs were found to be 0.08 and 0.15, respectively (Fig. 1). This could be explained by stabilization of droplets after the addition

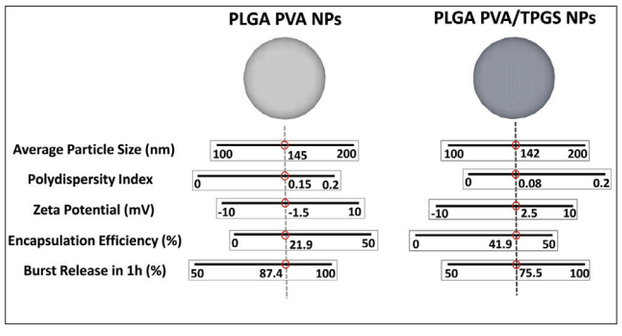


Fig. 1: Characteristics of PLGA PVA and PLGA PVA/TPGS NPs.

of TPGS. Also, burst release of ibuprofen within 1h from PLGA PVA/TPGS and PLGA PVA NPs were found as 75.5 and 87.4 %, respectively. Reducing of the drug release rate could be explained by dual functions of the surfactants. Surfactants act as a barrier in the internal interface and a steric stabilizer in the external interface in the nanoprecipitation process (Mora-Huertas et al. 2010). The change in Zeta potentials of NPs was not significant ($p>0.05$) (Fig. 1).

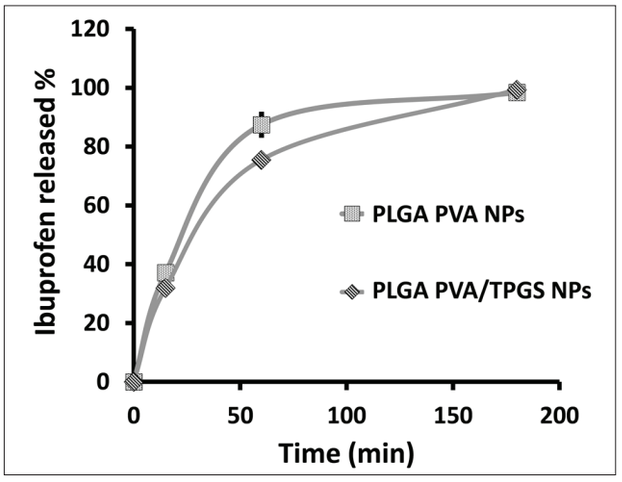


Fig. 2: Ibuprofen release from PLGA PVA and PLGA PVA/TPGS NPs.

In the FTIR spectrum, the main peaks of ibuprofen were displayed at 2700-3300 $cm^{-1}$ regions related to stretching vibrations of the C-H group bands in both PLGA PVA and PLGA TPGS/PVA NPs (Fig. 3) (Vueba et al. 2008). These peaks demonstrate that there was no interaction between ibuprofen and PLGA, PVA and TPGS. Besides, PVA peaks were present on PLGA PVA NPs as free and hydrogen bonded – OH bands at 3200 to 3600 $cm^{-1}$ respectively and they disappeared in PLGA TPGS/PVA NPs (Fig. 3).

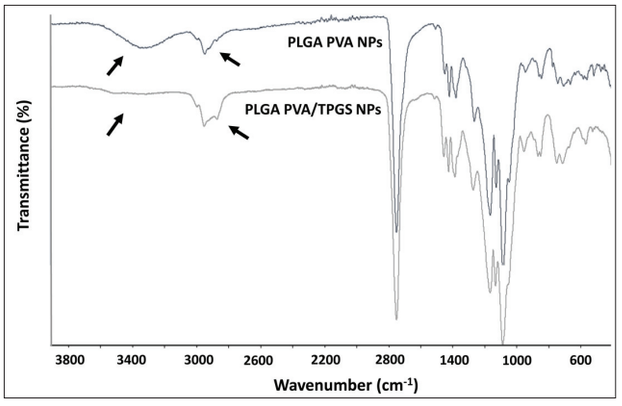


Fig. 3: FTIR spectrum of PLGA PVA and PLGA PVA/TPGS NPs.

In particle size measurement of NPs, Dynamic Light Scattering (DLS) is a frequently used method for particle size measurement of NPs with some limitations like occurrence of a hydration layer around the NPs in water (Cho et al. 2013). So this result should be corrected by microscopic analyses. For this reason, transmission electron microscopy (TEM) analysis was performed and the obtained images were evaluated. It was found that NPs have uniform particle size and NPs nearly spherical particle shape (Fig. 4).

In a previous study, it was reported that ibuprofen loaded PLGA NPs showed higher antiproliferative effects on Human MKN-45 cells than same concentration of ibuprofen in solution (Bonelli et al. 2012). In this study, for the evaluation of anticancer activity of improved ibuprofen loaded NPs, *in vitro* cytotoxicity of ibuprofen solution, PLGA PVA/TPGS and PLGA PVA NPs were compared with MCF-7 (human breast adenocarcinoma cells) and Caco-2 (human epithelial colorectal adenocarcinoma) cells. It was found that cytotoxicity of ibuprofen (400 µM) loaded PLGA PVA/TPGS NPs on MCF-7 and Caco-2 cells was significantly higher than that of ibuprofen (400 µM) solution and PLGA PVA/TPGS NPs were not cytotoxic on healthy cells (L929, mouse healthy fibroblast cells) when applied in the same concentration (Fig. 5). In conclusion, these results suggest that applying TPGS and PVA in combination as an emulsifier could be a promising approach to prepare

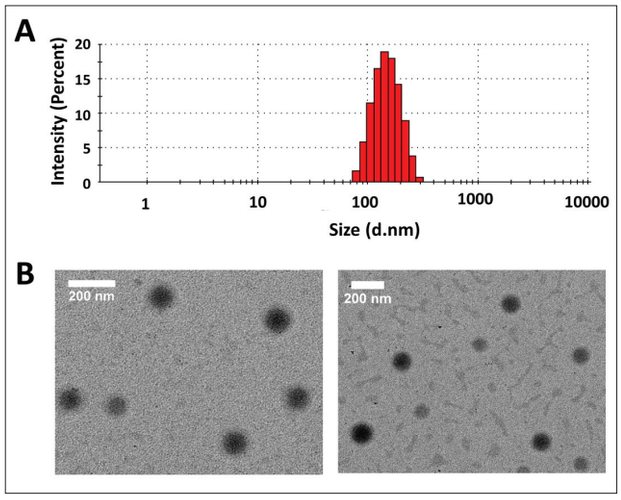


Fig. 4: Particles size distribution of PLGA PVA/TPGS NPs measured by DLS (A) and TEM images of PLGA PVA/TPGS NPs (B).

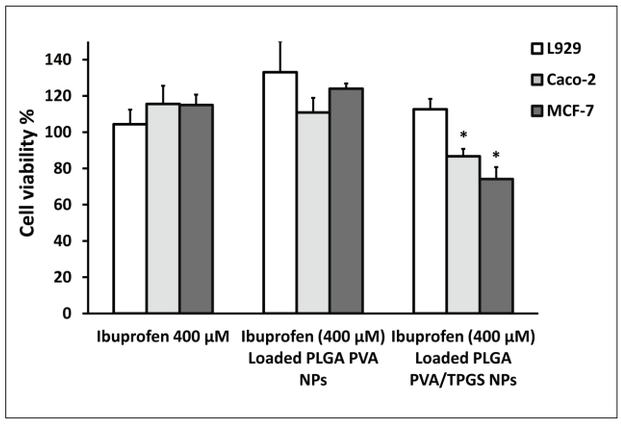


Fig. 5: Cytotoxicity of ibuprofen loaded PLGA PVA/TPGS NPs on L929, Caco-2 and MCF-7 cells at end of the 24h (n=6). (Student's *t-test* was used to comparison of ibuprofen loaded NPs and ibuprofen solution. * is indicated that cell viability was significantly decreased, $P<0.05$).

ibuprofen loaded PLGA NPs leading to improved characteristics and a better anticancer activity of NPs.

## 3. Experimental

### 3.1. Materials

Ibuprofen was donated by Drogsan Pharmaceuticals (Ankara, Turkey). PLGA RG503H were obtained from Boehringer Ingelheim (Ingelheim, Germany). PVA (87-90% hydrolyzed, average mol wt 30,000-70,000), acetone, acetonitrile, dimethyl sulfoxide (DMSO), TPGS, Cell WST-1 were obtained from Sigma Aldrich (MO, USA). All the reagents were of analytical or chromatographic grade.

### 3.2. Preparation of ibuprofen loaded PLGA NPs

Ibuprofen loaded PLGA NPs were prepared by nanoprecipitation (Barichello et al. 1999). 10 mg ibuprofen and 50 mg PLGA were dissolved in 4 mL acetone. Aqueous phase including various amounts of PVA or combination of PVA and TPGS were prepared (Table). The organic phase was added to the aqueous phase dropwise under magnetic stirring. The suspension obtained was stirred overnight to evaporate acetone. NPs were collected by centrifugation at 24.000 x g for 45 min and dispersed in ultrapure water. This process was repeated twice to wash NPs.

### 3.3. Characterization of NPs

#### 3.3.1. Average particle size and zeta potential

NPs' average particle size and zeta potential were measured using a Zetasizer Nano ZS (Malvern instruments, UK). These measurements were performed at 25 °C in ultrapure water.

#### 3.3.2. Encapsulation efficiency (EE)

For the determination EE of NPs, 1 mg of ibuprofen loaded PLGA NPs was dissolved in DMSO. The amount of ibuprofen was determined by high performance liquid chromategraphy (HPLC) equipped with an ultraviolet-visible (UV) detector (Agilent Technologies Inc., USA) at 222 nm. The mobile phase was composed of 20 mM phosphate buffer solution (pH 2.5) and acetonitrile in a volume ratio of 40:60. The injection volume was 30 µL and an Inertsil ODS-3 C18 (4.6 mm × 250 mm, 5 µm) analytical column was used in the analyses (Mannila et al. 2005). EE was calculated using the following equation:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of ibuprofen in yielded NPs}}{\text{Amount of ibuprofen which is added to formulation}} \times 100$$

#### 3.3.3. In vitro ibuprofen release from NPs

For the evaluation of *in vitro* ibuprofen release, the NPs and ibuprofen solution as a control were replaced in dialysis membrane and maintained in PBS medium containing tubes under sink conditions. The tubes were shaken at 37 °C under constant stirring at 100 rpm. Samples were withdrawn after 30, 60 and 180 min and the same volumes of fresh medium were added. Ibuprofen content in the samples was determined by HPLC-UV as described above.

#### 3.3.4. Transmission Electron Microscopy (TEM)

For determining the particle size and size distribution as well as the morphology and shape of PLGA PVA/TPGS NPs, NPs were suspended in ultrapure water and dropped onto the grid, dried and analyzed under a microscope (Tecnai G² Spirit Biotwin 20-120 kV, FEI Company, Netherland).

#### 3.3.5. Fourier-Transform Infrared (FTIR) spectra

To compare the molecular state of NPs, FTIR spectra of PLGA PVA and PLGA PVA/TPGS NPs were recorded on an ALPHA FTIR Spectrometer (Bruker, USA) using 30 scans with a resolution of 4 $cm^{-1}$ in 4000–400 $cm^{-1}$ wavenumber region.

### 3.4. Cytotoxicity of nanoparticles

Cytotoxicity of PLGA PVA/TPGS and PLGA PVA NPs on MCF-7 (human breast adenocarcinoma cells), Caco-2 (human epithelial colorectal adenocarcinoma) cells and L929 cells (mouse healthy fibroblast cells) was evaluated. Briefly, high glucose DMEM containing 10% (v/v) FBS, 50 U/mL–50 µg/mL penicillin-streptomycin, 2 mm l-glutamine was used as culture medium. $5 \times 10^3$ cells/well were added in 96-well plates and cells were incubated in at 37 ºC under 5% $CO_2$ for 24 h. Then the medium was removed and fresh medium which contained the formulation was added into the wells (n=6) and incubated for 24 h. WST-1 cell proliferation reagent (10 µL) was added into wells and after 2 h incubation at 37 °C, absorbance at 450 nm (reference at 650 nm) was measured by microplate reader (VersaMax, USA).

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Conflicts of interest: None declared.

### References

Barichello JM, Morishita M, Takayama K, Nagai T (1999) Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by the nanoprecipitation method. Drug Dev Ind Pharm 25: 471-476.

Baron JA (2003) Epidemiology of non-steroidal anti-inflammatory drugs and cancer. Prog Exp Tumor Res 37: 1-24.

Bonelli P, Tuccillo FM, Federico A, Napolitano M, Borrelli A, Melisi D, Rimoli MG, Palaia R, Arra C, Carinci F (2012) Ibuprofen delivered by poly(lactic-co-glycolic acid) (PLGA) nanoparticles to human gastric cancer cells exerts antiproliferative activity at very low concentrations. Int J Nanomed 7: 5683-5691.

Catarina Pinto Reis JPF, Candeias S, Fernandes C, Martinho N, Aniceto N, Silvério Cabrita A, Figueiredo IV (2013) Ibuprofen nanoparticles for oral delivery: proof of concept. J Nanomed Biother Discov 4 (119).

Cho EJ, Holback H, Liu KC, Abouelmagd SA, Park J, Yeo Y (2013) Nanoparticle characterization: state of the art, challenges, and emerging technologies. Mol Pharm 10: 2093-2110.

Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Preat V (2012) PLGA-based nanoparticles: an overview of biomedical applications." J Control Release 161: 505-522.

Dinarvand R, Sepehri N, Manoochehri S, Rouhani H, Atyabi F (2011) Polylactide-co-glycolide nanoparticles for controlled delivery of anticancer agents." Int J Nanomed 6: 877-895.

Duhem N, Danhier F, Preat V (2014) Vitamin E-based nanomedicines for anti-cancer drug delivery. J Control Release 182: 33-44.

Feng SS, Zeng W, Teng Lim Y, Zhao L, Yin Win K, Oakley R, Hin Teoh S, Hang Lee RC, Pan S (2007) Vitamin E TPGS-emulsified poly(lactic-co-glycolic acid) nanoparticles for cardiovascular restenosis treatment. Nanomedicine (Lond) 2: 333-344.

Harris RE (2015) Ibuprofen in the prevention and therapy of cancer. In: Rainsford KD (ed.) Ibuprofen, Discovery, Development and Therapeutics. John Wiley & Sons, Ltd, pp. 518-546.

Hillaireau H, Couvreur P (2009) Nanocarriers' entry into the cell: relevance to drug delivery. Cell Mol Life Sci 66: 2873-2896.
Kamaly N, Yameen B, Wu J, Farokhzad OC (2016) Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. Chem Rev 116: 2602-2663.
Langman MJ, Cheng KK, Gilman EA, Lancashire RJ (2000) Effect of anti-inflammatory drugs on overall risk of common cancer: case-control study in general practice research database. BMJ 320: 1642-1646.
Mannila A, Rautio J, Lehtonen M, Järvinen T, Savolainen J (2005) Inefficient central nervous system delivery limits the use of ibuprofen in neurodegenerative diseases. Eur J Pharm Sci 24: 101-105.
Martins AF, Follmann HD, Monteiro JP, Bonafe EG, Nocchi S, Silva CT, Nakamura CV, Girotto EM, Rubira AF, Muniz EC (2015) Polyelectrolyte complex containing silver nanoparticles with antitumor property on Caco-2 colon cancer cells. Int J Biol Macromo<u>l</u> 79: 748-755.
Mora-Huertas CE, Fessi H, Elaissari A (2010) Polymer-based nanocapsules for drug delivery. Int J Pharm 385: 113-142.
Mora-Huertas CE, Fessi H, Elaissari A (2011) Influence of process and formulation parameters on the formation of submicron particles by solvent displacement and emulsification-diffusion methods critical comparison. Adv Colloid Interface Sci 163: 90-122.
Saadati R, Dadashzadeh S (2014) Marked effects of combined TPGS and PVA emulsifiers in the fabrication of etoposide-loaded PLGA-PEG nanoparticles: in vitro and in vivo evaluation. Int J Pharm 464(1-2): 135-144.
Sah E, Sah H (2015) Recent trends in preparation of poly(lactide-co-glycolide) nanoparticles by mixing polymeric organic solution with antisolvent. J Nanomaterials 2015: 22.
Sahoo SK, Panyam J, Prabha S, Labhasetwar V (2002) Residual polyvinyl alcohol associated with poly (D,L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. J Control Release 82: 105-114.
Schubert S, Delaney JT, Schubert US (2011) Nanoprecipitation and nanoformulation of polymers: from history to powerful possibilities beyond poly(lactic acid). Soft Matter 7: 1581-1588.
Sun Y, Yu B, Wang G, Wu Y, Zhang X, Chen Y, Tang S, Yuan Y, Lee RJ, Teng L, Xu S (2014) Enhanced antitumor efficacy of vitamin E TPGS-emulsified PLGA nanoparticles for delivery of paclitaxel. Colloids Surf B Biointerfaces 123: 716-723.
Varan G, Oncul S, Ercan A, Benito JM, Ortiz Mellet C, Bilensoy E (2016) Cholesterol-targeted anticancer and apoptotic effects of anionic and polycationic amphiphilic cyclodextrin nanoparticles. J Pharm Sc<u>i</u> 105: 3172-3182.
Vij N, Min T, Bodas M, Gorde A, Roy I (2016) Neutrophil targeted nano-drug delivery system for chronic obstructive lung diseases. Nanomedicine 12: 2415-2427.
Vueba ML, Pina ME, Batista de Carvalho LA (2008) Conformational stability of ibuprofen: assessed by DFT calculations and optical vibrational spectroscopy. J Pharm Sci 97: 845-859.
Wicki A, Witzigmann D, Balasubramanian V, Huwyler J (2015) Nanomedicine in cancer therapy: challenges, opportunities, and clinical applications. J Control Release 200: 138-157.
Xu XC (2002) COX-2 inhibitors in cancer treatment and prevention, a recent development. Anticancer Drugs 13: 127-137.
Yerlikaya F, Ozgen A, Vural I, Guven O, Karaagaoglu E, Khan MA, Capan Y (2013) Development and evaluation of paclitaxel nanoparticles using a quality-by-design pproach. J Pharm Sci 102: 3748-3761.