Research Article **IJNR (2017) 1:1**

International Journal of Nanoparticle Research
(IJNR)

Cytotoxicity Studies on Naproxen and Piroxicam Nanoformulations

Sandeep Patnaik[a,b], L.A. Avinash Chunduri[a,b], Aditya Kurdekar[b], Venkataramaniah Kamisetti[b*]

[a]Andhra Pradesh Medtech Zone Ltd., Vishakhapatnam, India
[b]Laboratories for Nanoscience and Nanotechnology(LNAN) Research, Department of Physics, Sri Sathya Sai Institute of Higher Learning, Prasanthinilayam, India

ABSTRACT

Caco-2 cells were used as in vitro models to assess the cell viability characteristics of the carriers Soluplus®, Gelucire 50/13 and PVP K25 and the nanoformulations of Naproxen and Piroxicam. The assessment of cell viability was done using the tetrazolium salt based MTT assay. Gelucire 50/13 and its NFs were observed to have slightly higher cytotoxicity than PVP and Soluplus® and their respective NFs. All the NFs were observed to follow the cytotoxicity trend of the polymers. Our results show that no significant decrease in cell viability was seen until 0.01% concentration of Gelucire 50/13 for 12-h exposure. The NFs as well as the polymers alone had no significant effect on the viability of Caco-2 cells below 0.01% concentrations. The intestine has a protective mucous layer, whereas the cell culture monolayers do not. The intestinal tissues also have more capacity to recover from trauma than the cultured cells. Hence the present NFs can be expected to show lesser cytotoxicity when subjected to in vivo studies.

Keywords: Caco-2 cells, Cytotoxicity, Nanoformulations, Polymers, MTT assay

***Correspondence to Author:**
Venkataramaniah Kamisetti
Laboratories for Nanoscience and Nanotechnology(LNAN) Research, Department of Physics, Sri Sathya Sai Institute of Higher Learning, Prasanthinilayam, India

How to cite this article:
Sandeep Patnaik, L.A. Avinash Chunduri, Aditya Kurdekar, Venkataramaniah Kamisetti.Cytotoxicity Studies on Naproxen and Piroxicam Nanoformulations. International Journal of Nanoparticle Research, 2017; 1:1.

eSciencePublisher
eSciPub LLC, Houston, TX USA.
Website: http://escipub.com/

Introduction

Nanotechnology involves the creation and manipulation of materials at nanoscale levels to create products that exhibit novel properties. There are important applications of nanoscience in biology and biotechnology, and nanotechnology offers new tools to biologists1. Nevertheless, despite the increased interest in the development of nanoparticles, few studies address their potential toxicity. The rapidly developing field of nanotechnology is likely to become yet another source of human exposure to nanoparticles by different routes: inhalation, ingestion, dermal, and injection. Regulatory agencies, researchers, and health and environmental watchdogs are assessing how nanoscale materials affect human health and the environment2. Similarly, the characteristic biokinetic behavior of nanoparticles is an attractive quality for promising applications in medicine. Such applications include diagnostic and therapeutic devices and tools to investigate and understand molecular processes and structures in living cells. However, in stark contrast to the many efforts aimed at exploiting the desirable properties of nanoparticles for improving human health, attempts to evaluate potential undesirable effects when administered for medical purposes or after exposure during manufacture or processing for industrial applications are limited.

Nanotoxicology, an emerging discipline, is gaining increased attention. Nanotoxicology research will not only provide data for safety evaluation of engineered nanostructures and devices, but will also help to advance the field of nanomedicine by providing information about their undesirable properties and means to avoid them2. The safety and toxicity of nanoparticles are of growing concern despite their significant scientific interest and promising potential in many applications. Their biological activity and biokinetics are dependent on many parameters: size, shape, chemistry, charge, surface modifications, etc. When inhaled, they can translocate out of the respiratory tract via different pathways and mechanisms. When ingested, systemic uptake of nanoparticles via lymph can occur. When in blood circulation, they can distribute throughout the organism, and they are taken up by liver, spleen, bone marrow, heart, and other organs such as testis. The study of the toxic effect of nanoparticles on gametogenesis is of great interest. The identification of toxic properties of new nanodrug formulations at an early stage has a high priority before human or animal testing in vivo can begin.

Despite many established bioconjugation strategies for targeting purposes with nanoparticles, concerns regarding their clinical success have risen. Recently, it has been shown that the targeting capability and stability of bioconjugated NPs may disappear when they are placed in a biological environment. The primary reason is the presence of a complex mixture of distinct proteins in the biological media. Prior to cellular uptake, these proteins adsorb rapidly onto the surface of NPs, leading to the formation of a so-called “corona”, which may obscure specific recognition of bioligands on the NP surface and hamper their targeting applicability. Indeed, at physiological conditions, biological systems are often exposed to NP-protein corona complexes, which differ significantly from bare NPs and define surface properties, aggregation rate, and hydrodynamic size of NPs.

Materials and Methods

Synthesis of Naproxen Nanoformulations

The NFs were prepared via wet milling using a conventional Retsch Planetary ball mill in various ratios of drug to polymer (1:1, 1:2, 1:3, 1:4). The drug and polymer (in the required ratios) were introduced into an agate milling chamber containing 1 mm agate balls. 40 mL of 0.5% aqueous solution of Tween 80 was added to fill the chamber. The samples were co-milled at 500 rpm for 6 hours. Regular breaks of 5 minutes were provided after every 15 minutes of milling to avoid overheating caused due to the

high energy involved in the milling process. The nanosuspensions thus formed by co-milling were lyophilized for about 24 hours and gently powdered to obtain free flowing powders. To protect the nanoparticles from damage, due to ice formation and to minimize the particle size growth during lyophillization, mannitol (0.1% by weight) was added as a cryoprotectant prior to lyophillization.

Synthesis of Piroxicam Nanoformulations

Nanodispersions were prepared with drug to PVP (K-25)/Soluplus in the weight ratios 1:1,1:2, 1:3, 1:4 and 1:5 by means of the solvent evaporation method. To a solution of piroxicam in acetone (0.5 g in 30 ml), the appropriate amount of PVP was added while stirring over magnetic stirrer. The minimum amount of isopropyl alcohol (IPA) was added to solubilize the polymer PVP (for Soluplus, IPA was not necessary). These solvents were chosen because they are less toxic and are considered safer (Class 3) according to US-FDA ICH guidelines. The solvents were removed under reduced pressures of a vacuum rotary evaporator at 40 °C and dried under vacuum at about 80 oC for 2 days and then carefully stored in a vacuum desiccator. The samples were pulverized using a mortar and pestle, and the 0.05–0.25 mm particle size fractions were obtained by sieving with a sieve size of about 250 microns (50 BSS units).

Rotary evaporation is a very simple, economical and robust method for solvent removal during prepration of nanodispersions for early stage and laboratory scale studies. Since the evaporation time is relatively long in the process, the drug and carriers must be sufficiently stable in the solvent at the temperatures used. For our experiments, since the solvent used was predominantly acetone (boiling point 56 oC), the bath temperature was set to 30 oC and the vacuum was set to 25 mm Hg.

Physico-chemical Characterization of Naproxen nanoformulations

FTIR Analysis

FTIR spectra of pure naproxen, all the NFs and the carriers were recorded using an FTIR Spectrophotometer (Spectrum FTIR (Scimadzu, IRAffinity-1)) in the range of 4000–400 cm−1. The sample was in KBr followed by gentle mixing. The spectrum was scanned at a resolution of 0.15 cm−1 and scan speed was 20 scans per second.

As can be seen in Figure 1, the polymer Soluplus® showed peaks at 3450 cm−1 (O-H stretching), 2924 cm−1 (aromatic C-H stretching), 1736 cm−1 , 1635 cm−1 (C-O stretching), and 1477.21 cm−1 (C-O-C stretching). The carbonyl peaks of the vinyl acetate (VAC) and vinyl caprolactam (VCL) is located at 1733cm-1 and 1634 cm−1, respectively. The VCL carbonyl band can be observed to be split into two distinct bands at 1634cm-1 and 1595 cm−1. Considering that carbonyl absorption bands are shifted to lower wavenumbers when H-bonds are formed, the new band at 1595 cm−1 can be assigned to the VCL component that is H-bonded to the drug. This finding suggested that naproxen interacted with Soluplus®, predominantly by hydrogen bonding. It is important to mention here that this kind of interaction between drug and carrier is an additional benefit for the nanoformulations, since besides increasing the solubility of the drug in the carrier they would also inhibit the (re)crystallization of the drug.

XRD Analysis

As evident from Figure 2, the spectrum of the Soluplus® contains broad indistinct peaks resulting from the anisotropic scattering of X-rays indicating its amorphous nature, while distinct peaks of naproxen appeared at 14.50, 17.73 and 27.45. The X-ray spectra of the nanoformulations are observed to show reduction in the intensity of diffraction peaks. This reduction in the intensity of peaks compared to pure naproxen indicates the decrease in crystallinity or partial amorphization of the drug in the NS 1 and NS 2 formulations. NS 4 formulation showed absence of any of the crystalline peaks of naproxen indicating that

complete amorphization was achieved at this drug to carrier ratio.

FESEM Analysis

Figure 3 shows the FESEM images of two representative formulations corresponding to low (NS 1) and high polymer content (NS 4) at different magnifications. Both the formulations

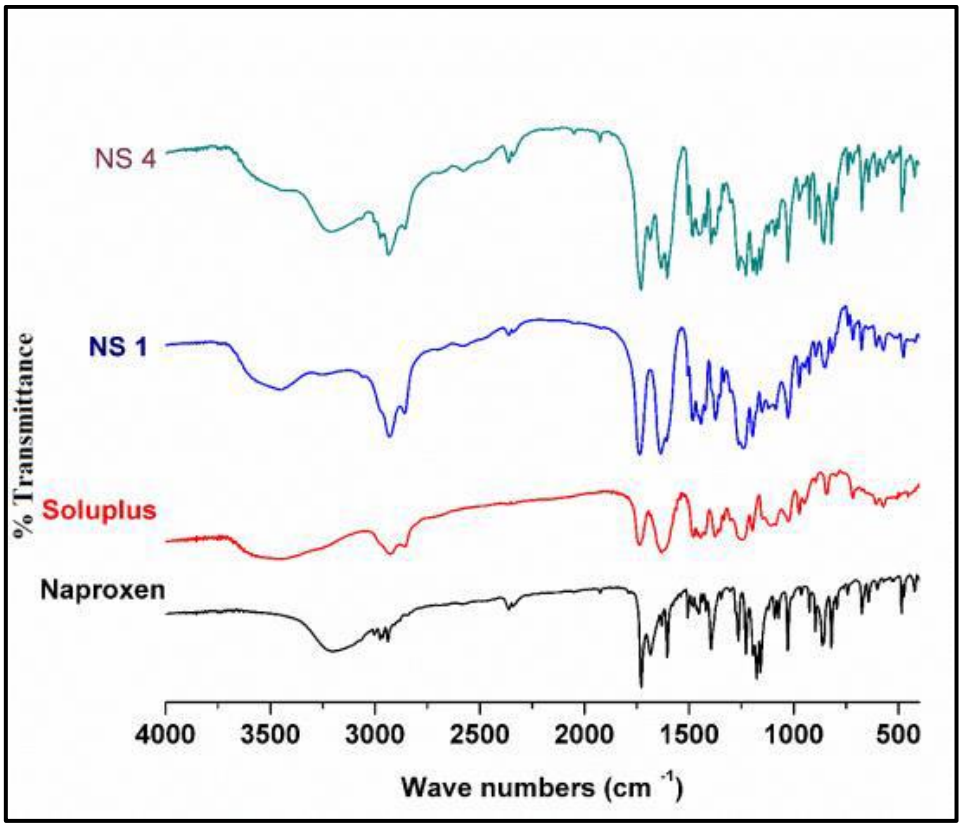

Figure1: FTIR spectra of Naproxen,

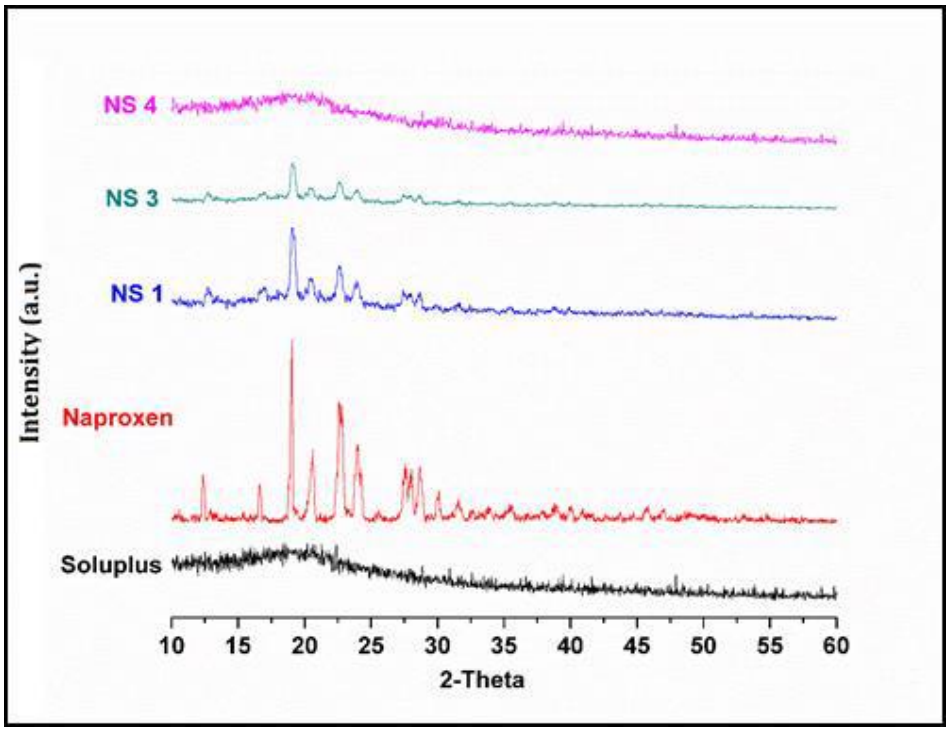

Figure 2: XRD spectra of Naproxen, Soluplus and the nanoformluations Soluplus and the nanoformluations

showed irregular morphologies. Since ball milling is brute force top down approach it offered little control over the particle size distribution (PSD) for both the formulations. NS 1 showed smooth rounded irregular particles while NS 4 showed more or less irregular shapeless mossy morphology probably due to presence of the excess amount of polymer.

Physicochemical Characterization of Piroxicam nanoformulations

XRD Analysis

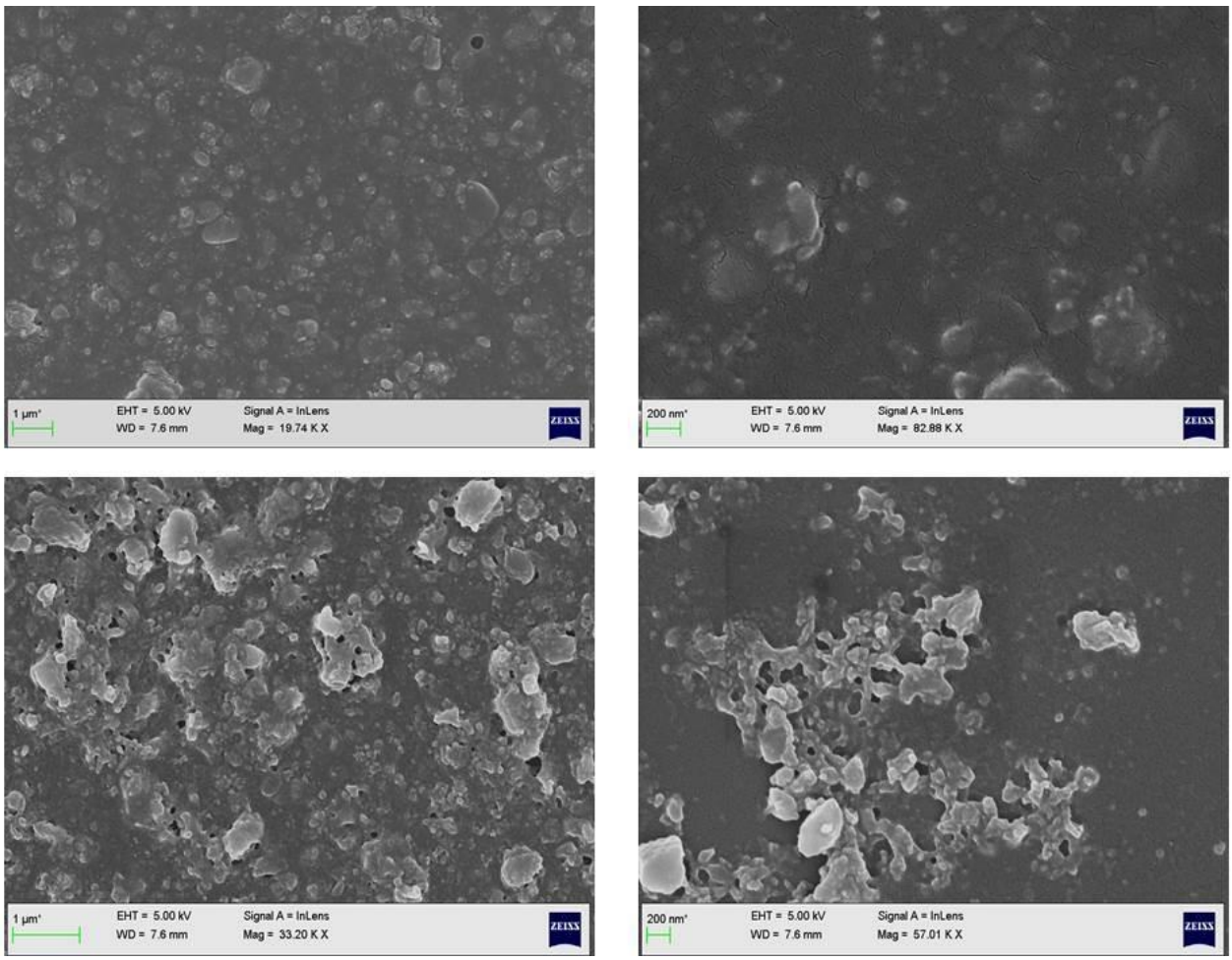

Figure 3: FESEM images showing the morphologies of Naproxen- Soluplus Nanoformualtions NS1 (Top) and NS 4 (down).

XRD patterns were recorded using PANalytical X'pert Pro MPD diffractometer, with the following settings: Cu Kα radiation with wavelength 1.54 Å, voltage = 45 kV, current = 40mA. Measurements were made in the 2θ range of 10 to 80o. It can be noted that the polymers, Soluplus® and PVP (Figure 4), are amorphous powders having no crystalline structure. However, for pure piroxicam crystalline peaks were observed at at the diffraction angles 8.89, 15.7, 23.0 and 25.9. The XRD patterns of the nanodispersions were clearly different from those of the crystalline piroxicam. With an increase in the composition of the polymeric species in the dispersions, the crystallinity was found to successively decrease. The crystalline peaks, corresponding to the API, completely disappeared for the dispersions of the compositions 1:5, in case of PVP, and 1:4 in case of Soluplus®, indicating that at these ratios the drug is entrapped as high energy amorphous state in the polymeric matrices.

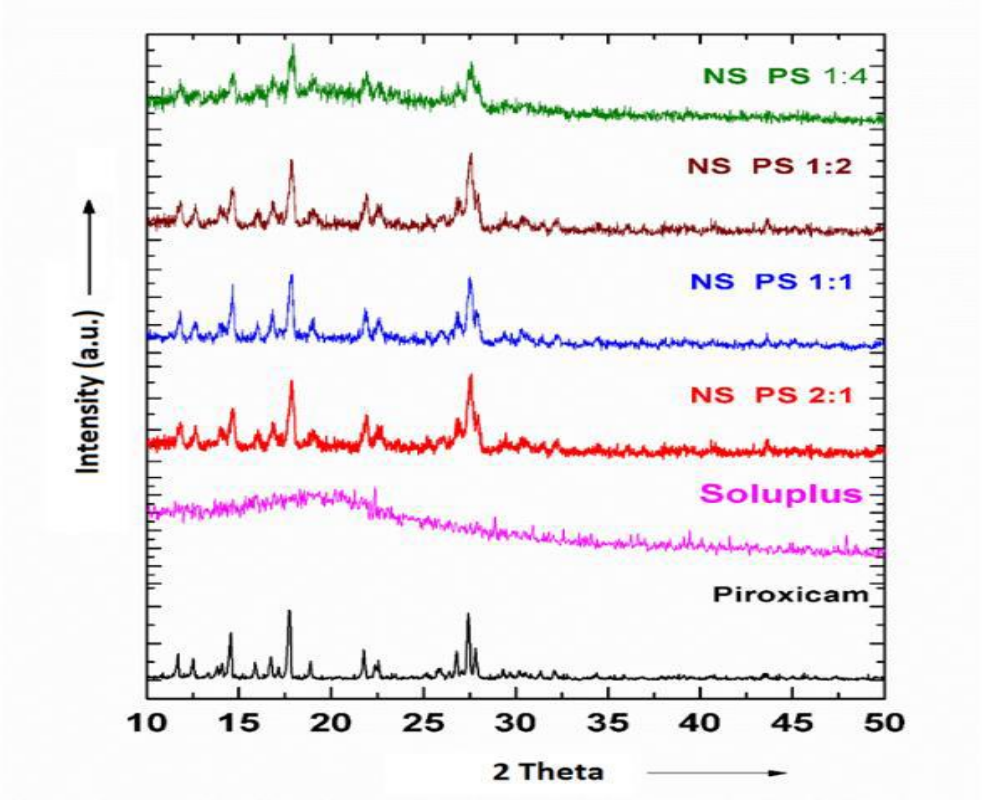

Figure 4: Overlay of the XRD spectra of Piroxicam,polymer Soluplus® and the nanodispersions in various ratios

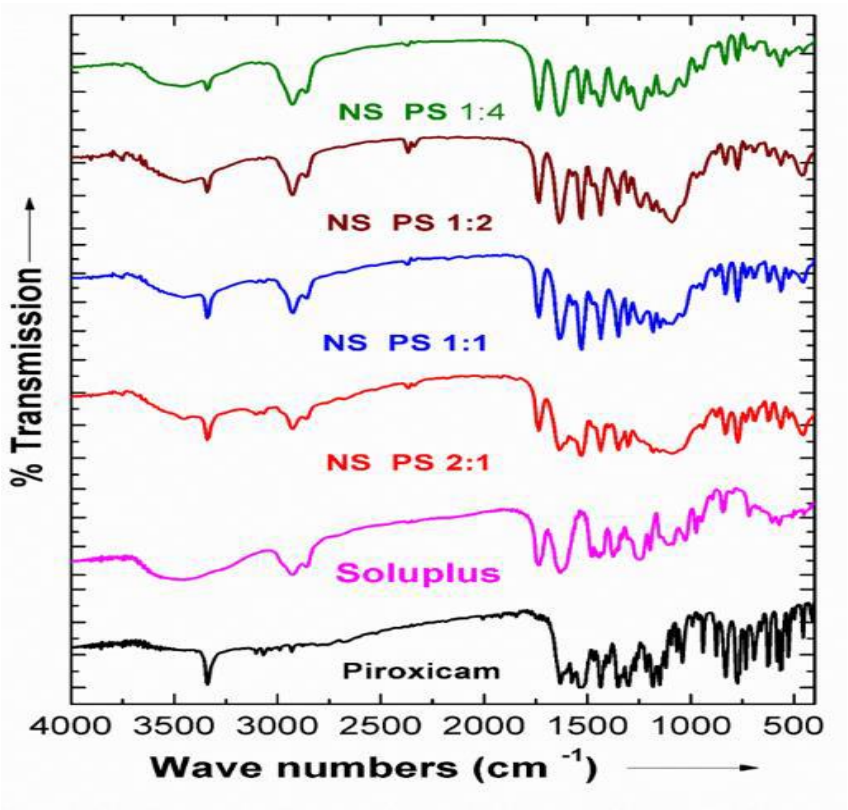

Figure 5: Overlay of the FTIR spectra of Piroxicam, polymer Soluplus® and the nanodispersions in various ratios.

FTIR Analysis

From Figures 4 and 5, it can be seen that the drug Piroxicam contains a hydrogen donor (–NH), whose characteristic stretching absorption peak is observed at 3339 cm-1. The spectrum of PVP portrays a broad absorption band at 3500cm-1 that is indicative of the –NH stretching absorption. The carbonyl stretching vibration was observed as a broad band at 1650- 1700 cm-1. In the spectrum of Soluplus®, a distinct, broad -OH peak was observed at 3300 to 3600 cm-1, The carbonyl stretching of the amide occurred at 1650 cm-1 while the carbonyl of the acetate gave an absorption peak at 1750. The peak at 2900-3000 can be attributed to the –NH stretching vibrations. The –C-O-C- bending vibrations, gave a peak at 1470. Nanodispersions also showed slightly different FTIR spectra in the fingerprint regions, the substantial differences were shown in the N-H or O-H stretching regions. As the proportion of polymers is increased, the broadening of the -OH peak occurred. The drug: PVP 1:1 showed doublets at 3341 and 3322 cm-1. The single absorption bands at 3337 cm-1 were observed in the FTIR spectra of the drug: PVP 1:2, 1:3 and 1:5. The complete loss of the N-H / O-H stretching vibration peaks of pure piroxicam in the spectra of ND 1:4, in case of Soluplus and ND 1:5 in case of PVP, suggest that the amine hydrogen of piroxicam has hydrogen bonded with the polymer, thus weakening the peak at 3339cm-1. This is in coherence with the XRD data.

FESEM Analysis

The SEM images of the nanoformulations of piroxicam and soluplus® in the ratios of 2:1 and 1:4 obtained from wet ball milling after 6 hours are shown in Figure 6. The particles were of varied morphology with particle shapes ranging from irregular, cuboidal to round. The size distribution varied from about 50nm to 300nm. Since ball milling is top down synthetic route, it offers poor control over morphology and size distribution. Both the formulations showed similar characteristics in terms of morphology and particle size.

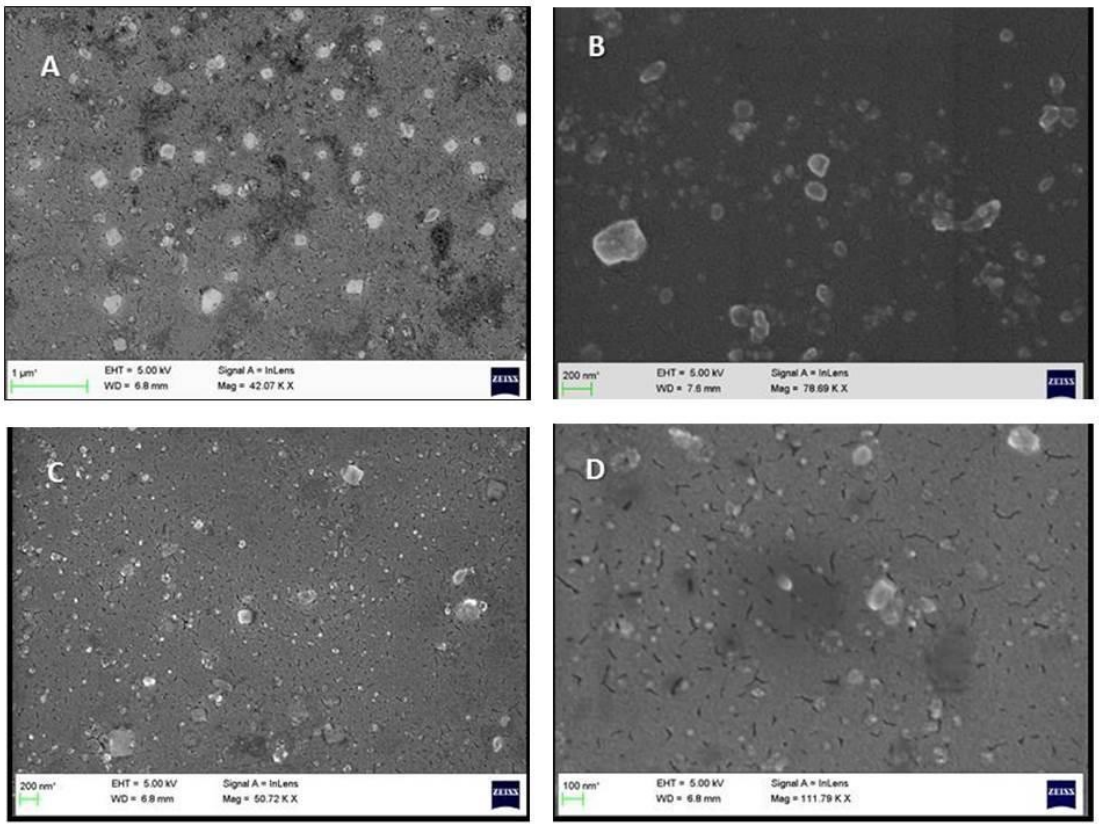

Figure 6: FESEM images of Nanodispersions of Piroxicam with PVP(A and B) and Soluplus.(C and D)

Cytotoxicity studies:

Caco-2 Cell Line

The Caco-2 cells are epithelial like continuous cell lines from human epithelial colorectal adenocarcinoma cells, originally developed by the Sloan-Kettering Institute for Cancer Research by Dr. Jorgen Fogh3. These are heterogenous adherent cell lines derived from human large intestine or colon carcinoma. On culturing under specific conditions, these cells differentiate and get polarized in a manner that their phenotype bears a resemblance to that of the enterocytes of the small intestine4-6. Caco-2 cells express tight junctions, microvilli, and a number of enzymes and transporters that are specific of enterocytes: peptidases, esterases, P-glycoprotein, uptake transporters for amino acids, bile acids carboxylic acids, etc. When looking at Caco-2 cell cultures microscopically, it is evident even by visual inspection that the cells are heterogeneous.

Caco-2 cells are most commonly used not as individual cells, but as a confluent monolayer on a cell culture insert filter. When cultured in this format, the cells differentiate to form a polarized epithelial cell monolayer that provides a physical and biochemical barrier to the passage of ions and small molecules. The Caco-2 monolayer is widely used across the pharmaceutical industry as an in vitro model of the human small intestinal mucosa to predict the absorption of orally administered drugs. The correlation between the in vitro apparent permeability across Caco-2 monolayers and the in vivo fraction absorbed is well established. The Caco-2 cell culture model is not only a rapid screening tool for drug absorption studies but, for the purpose of this study, is a tool to measure mucosal toxicity caused by excipients such that their influence on membrane barrier properties and, ultimately, the mechanism of drug absorption can be understood6

Caco-2 Culture and Cytotoxicity Protocol

Culture Medium: Eagle's Minimum Essential Medium (pH 7.4)

Other Additives: Heat inactivated foetal bovine serum (Gibco,Invitrogen), sodium bicarbonate, antimycotic and antibacterial mixture and gentamycin

Passage Number: 12 – 25

Period of Cell Culture: 20 days

Number of Replicates: 3

Test Compound Concentration: 10 μM

Incubation Time: 2 hours

Temperature: 37° C

Assay type: Cell viability, MTT assay

Compound Requirements: 100 μL of 10 mM DMSO solution

Analysis Method: SpectraMax M 5, Molecular devices quantification

Cell culture experiments

The cell culture experiments were performed in a biosafety level II cabinet hood. The Caco-2 cells of passage number 12-25 were cultured using minimum essential medium, MEM (pH 7.4) in culture plates at 37 oC in a CO2 incubator. The other supplements added to the medium were heat inactivated foetal bovine serum, sodium bicarbonate, antimycotic and antibacterial mixture and gentamycin. The medium was changed every alternate day till the cells became 80% confluent. The washing of the cells were done using phosphate buffer saline and the removal of cells were performed by trypsinization (0.25% trypsin,0.2% EDTA solution) at 37 oC for 7-10 mins. The cells obtained from trypsinization were obtained as a pellet by centrifugation for 3 minutes at 200 x g and resuspended into the MEM and used for sub-culturing or cytotoxicity studies.

A population of cells that have descended from a single parent cell and are comprising of the same genetic makeup is termed as a cell line. Cell lines are, thus derived from primary culture and have a limited life span. Once the cells are grown and have achieved a confluency of about 90%-100% (varies from cell line to cell line), the cells are passaged or split. As they are passaged, cells with the highest growth capacity

predominate, resulting in a degree of genotypic and phenotypic uniformity in the population. The passage number is an important cell line parameter and needs to be recorded. It indicates the approximate number of cell divisions the culture has undergone. Microorganisms have a tendency to adapt to any new culture conditions, which is rarely as precise as the microorganisms natural environment. Hence their biology changes with increasing passages. In mammalian cells lines, with subsequent subculturing, chromosomal aberrations tend to increase with time. Ideally, the passage numbers of 8-20 are used for experiments.

MTT assay

A cell viability assay determines the ability of cells to maintain or recover viability7,8. Several markers are used to assess the effect of metabolites/drugs on the viability of cells. Cell-based assays are often used for screening collections of compounds to determine if the test molecules have effects on cell proliferation or show direct cytotoxic effects that eventually lead to cell death. These assays are also useful for measuring receptor binding and a variety of signal transduction events that may involve the expression of genetic reporters, trafficking of cellular components, or monitoring organelle function. Regardless of the type of cell-based assay being used, it is important to know how many viable cells are remaining at the end of the experiment. There are a variety of assay methods that can be used to estimate the number of viable eukaryotic cells. The MTT assay is a colorimetric cell viability assay used for assessing cell metabolic activity. It is based on enzymes called the NAD(P)H-dependent cellular oxidoreductase. These enzymes have the capacity to reduce the tetrazolium dye, MTT or 3-(4,5-dimethylthiazol-2-yl) -2,5 -diphenyltetrazolium bromide which is a pale yellow colored tetrazole, to its insoluble formazan, which is purple in color. The reduction of this tetrazolium dye would depend upon the amount of NAD(P)H-dependent oxidoreductase enzymes that are present predominantly in the cytosolic compartment of the cell. This in turn is governed by the cellular metabolic activity due to NAD(P)H flux. Cells with low metabolism rates (such as thymocytes and splenocytes) would be able to reduce very little MTT. On the contrary, rapidly dividing cells can be expected to exhibit higher rates of MTT reduction due to the presence of higher amount of the enzymes.

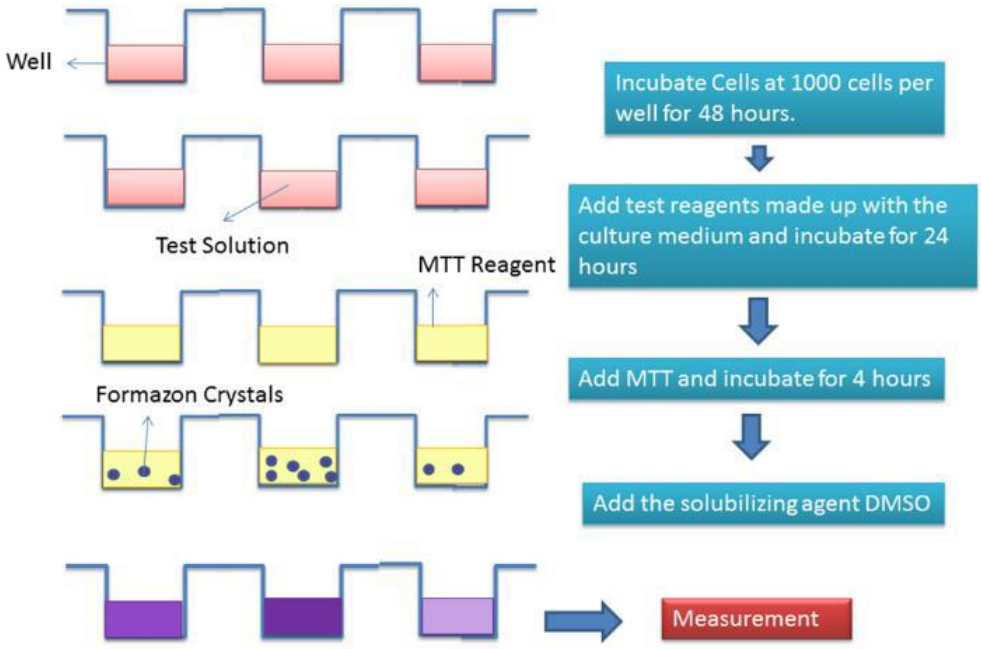

Figure 7: Flow chart highlighting the procedural protocol involved in MTT assay.

The resuspended cells obtained after trypsin treatment of an 80% confluent plate were seeded at a cell density of 4x103 in a 96 well plate for 48 hours and successively exposed to different concentrations of the carriers and the NFs made by the culture medium. Culture medium was changed after 48 hours of incubation and the test solutions were added. The cytotoxicity was assessed from the cell viability data obtained from the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazonium bromide] cell proliferation assay. After 48 hours of incubation, 20µL of the MTT reagent was added to each well. The plates were then incubated for another 4 hours. Then the medium was removed and the intracellular formazan was solubilized with 100mL of dimethyl sulfoxide (DMSO). The absorbance was recorded using a microplate reader (Spectramax M5) at 490 nm. Cell viability was assessed as the percentage of absorbance of the analyte with respect to the control. The statistical significance of the difference with respect to control was investigated by the two-tailed Student's t-test.

Cytotoxicity of Naproxen Nanoformulations

Caco-2 cell lines are often used as models to establish the membrane permeability of drugs. Gelucire 50/13 and its NFs were observed to have significantly higher cytotoxicity than PVP and Soluplus® as it showed only 50% cell viability at 0.5% concentration. There is an apparent increase in cell viability at 0.01% concentration of all the carriers beyond at 0.01% concentrations. This observed increase in the viability could be partly due to increased transport of tetrazolium salt inside the cells. A

Figure 9 shows the percent cell viability with respect to the control on exposure of various concentrations of the nanoformulations of Naproxen with the three carriers. They showed about 80% cell viability at 0.5% concentrations. The cytotoxicity profiles of the nanoformulations followed the trend of the polymers. The formulations NG 4 showed significant

Naproxen is a BCS class II drug and so it has excellent permeability across the GI membrane [126 (±4) X 106 cm/s]. In order to provide a comparative account on the cytotoxicity profiles of the carriers used and NFs the Caco-2 cells were exposed to the polymers and the NFs for 12 hours and the viability of the cells was assessed using the MTT assay. To evaluate and compare the contribution of the carriers to the cytotoxicity, assay was carried out with the formulations with the highest percentage of carrier (drug to carrier ratio 1:4). While the polymers were tested as aqueous solutions because they readily dissolve in water, the NFs were tested in their solutions with DMSO20. A 12-hour exposure time was selected for the present studies because scintigraphic gastric transit studies in humans suggest that the maximum exposure time of a drug in the gastrointestinal tract as 12 hours.

Figure 8 shows the percent cell viability with respect to the control on exposure of various concentrations of the carriers Soluplus®, Gelucire 50/13 and PVP. The carriers Soluplus® and PVP showed similar cell viability profiles. They showed about 80% cell viability at 0.5% concentrations.

similar phenomenon has also been observed in the case of other excipients as well21,22.Considering 80% cell viability as the benchmark22, PVP had no significant effect on the viability of Caco-2 cells below 0.5% concentrations while Soluplus® could keep the cells viable only at and below 0.1% concentration. Gelucire 50/13 remained toxic even at 0.1% concentrations.

cytotoxicity even at 0.1% concentrations while the formulations NS 4 and NP 4 could maintain 60% cell viability of Caco-2 cells even at 0.5% concentrations. As in the case of the carriers, the nanoformulations too triggered an apparent increase in cell viability at 0.01% concentrations which could be partly due to increased transport of tetrazolium salt inside the cells.

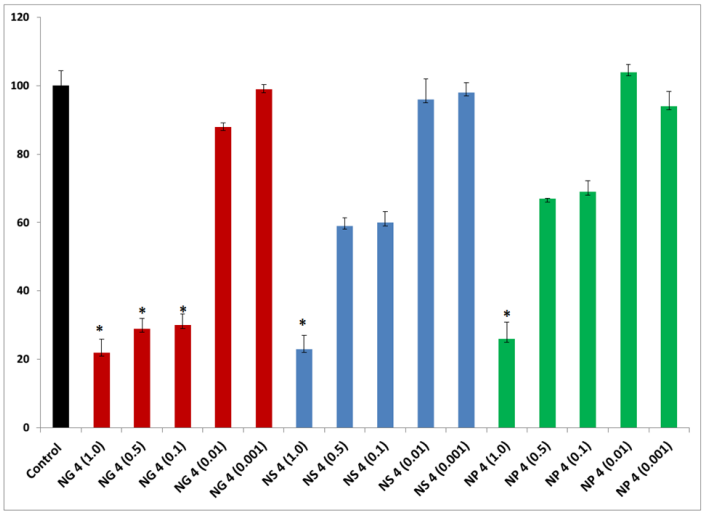

Figure 8: Cell Viability Data obtained from MTT assay on Caco 2 cells after 12 hours exposure to various carriers. S = Soluplus, G= Gelucire, PVP= Polyvinyl Pyrrolidone

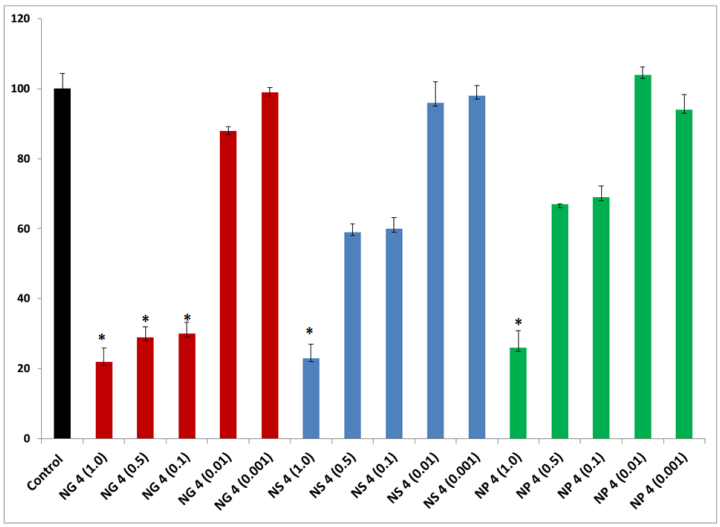

Figure 9: Cell viability data obtained from MTT assay performed on Caco-2 cells after 12 hour exposure to different nanoformulations of naproxen.

Cytotoxicity of Piroxicam-Soluplus® Nanoformulations

Figure 10 shows the percent cell viability (with respect to the control) on exposure of various concentrations of the carrier soluplus® and the piroxicam-soluplus® nanoformulations, NS PS 1:4 and NS PS 1:1. As discussed, Soluplus® showed about 80% cell viability at 0.5% concentrations. Significant decrease in cell viability was seen only above 0.1% of Soluplus for 12 hour exposure. The nanoformulations showed more cytotoxicity towards the Caco-2 cells than the pure polymer alone. At 0.5% concentrations, NS PS 1:4 maintained the viability of the Caco-2 cells at about 60%, while NS PS 1:1 could maintain the viability at only 40%. Therefore, NS PS 1:4 (NF with more carrier content) can be considered to have lower cytotoxicity to Caco-2 cells than NS PS 1:1. From this observation, it can be concluded that the polymeric carrier, Soluplus® 300 not only improves the dissolution characteristics but also reduces the cytotoxicity of the drug. The nanoformulations had no significant effect on the viability of Caco-2 cells below 0.01% concentrations. Here it is important to note that intact intestinal membranes (in vivo) often are found to be more resistant to the cytotoxic effects of excipients than are cell culture models (in vitro). The intestine has a protective mucous

layer, whereas the cell culture monolayers do not. The intestinal tissues also have more capacity to recover from trauma than the cultured cells. Hence the present nanoformulations can be expected to show lesser cytotoxicity in in vivo studies.

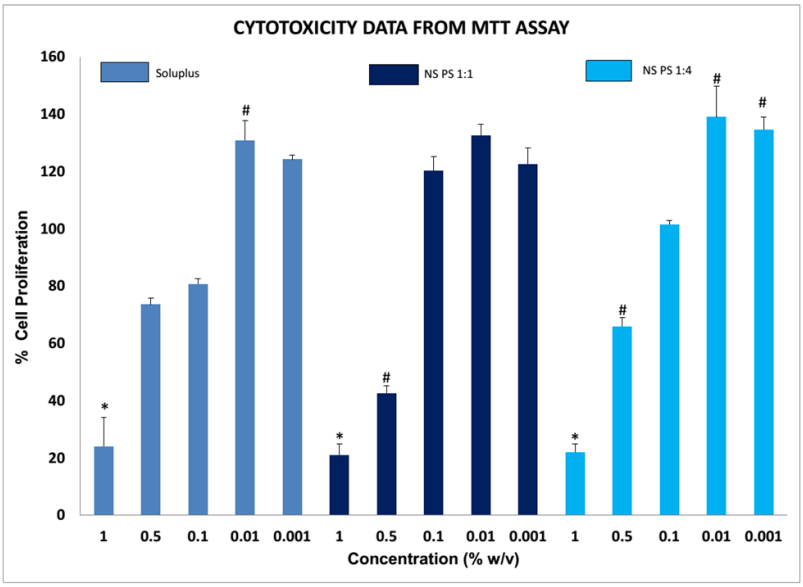

Figure 10: Cell viability data obtained from MTT assay of Caco-2 cells on exposure to the polymer Soluplus and the various nanoformulations of piroxicam.

Conclusions

Caco-2 cells were used as *in vitro* models to assess the cell viability characteristics of the carriers Soluplus®, Gelucire 50/13 and PVP K25 and the nanoformulations of Naproxen and Piroxicam. The assessment of cell viability was done using the tetrazolium salt based MTT assay. Duration of 12 hour exposure was selected because scintigraphic gastric transit studies in humans suggest they are physiologically relevant average and maximum concentrations. Here it is important to note that intact intestinal membranes (in vivo) often are found to be more resistant to the cytotoxic effects of excipients than are cell culture models (in vitro). The intestine has a protective mucous layer, whereas the cell culture monolayers do not. The intestinal tissues also have more capacity to recover from trauma than the cultured cells. Hence the present NFs can be expected to show lesser cytotoxicity when subjected to in vivo studies.

exposure times, respectively, in the gastrointestinal tract. Gelucire 50/13 and its NFs were observed to have slightly higher cytotoxicity than PVP and Soluplus® and their respective NFs. All the NFs were observed to follow the cytotoxicity trend of the polymers. Our results show that no significant decrease in cell viability was seen until 0.01% concentration of Gelucire 50/13 for 12-h exposure. The NFs as well as the polymers alone had no significant effect on the viability of Caco-2 cells below 0.01%

References

1. Scherer, W. F., Syverton, J. T. & Gey, G. O. Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix. J. Exp. Med. 97, 695–710 (1953).

2. Norrby, E. & Prusiner, S. B. Polio and nobel prizes: Looking back 50 years. Ann. Neurol. 61, 385–395 (2007).

3. Fogh, J., Fogh, J. M. & Orfeo, T. One Hundred and Twenty-Seven Cultured Human Tumor Cell Lines Producing Tumors in Nude Mice. J Natl Cancer Inst 59, 221–226 (1977).

4. Sambuy, Y. et al. The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. Cell Biol. Toxicol. 21, 1–26 (2005).

5. Arturssona, P., Palm, K. & Luthmanb, K. Caco-2 monolayers in experimental and theoretical drug transport predictions of. (1996).

6. Artursson, P. & Borchardt, R. T. Intestinal drug absorption and metabolism in cell cultures: Caco-2 and beyond. Pharmaceutical Research 14, 1655–1658 (1997).

7. Johnson, S., Nguyen, V. & Coder, D. Assessment of cell viability. Curr. Protoc. Cytom. (2013). doi:10.1002/0471142956.cy0902s64

8, Riss, T. L. et al. Cell Viability Assays. Assay Guid. Man. 1–23 (2004). doi:10.1016/j.acthis.2012.01.006