Production of NSAID pharmaceutical molecular liquids via RESS processing

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ABSTRACT

We report the production of pharmaceutical molecular clusters of most commonly implemented and prominent drug molecules such as ibuprofen and naproxen, via Rapid Expansion of Supercritical Solution (RESS) processing under mild conditions. These drug molecular clusters were first trapped in "dry ice" and subsequently solubilized in DI water under ambient conditions. These solutions are found to be stable over a period of several weeks. Drop casting and ambient drying of these solutions on a silicon substrate resulted in stable, viscous liquid films. Optical and electron microscopy showed typical liquid like behavior of these drop casted films. In-vitro cell viability studies, clearly demonstrated that the water-solubilized ibuprofen and naproxen exhibit similar cytotoxicity to the as received, raw drug powders thus retaining their original potency.

Keywords: supercritical fluids, ibuprofen, RESS, micronization, drug nanoparticles,

1 INTRODUCTION

In the pharmaceutical and drug development industry, one of the major unsolved problem is the poor aqueous solubility. The bioavailability of the drugs are limited by this insolubility. The drug must first be dissolved in order to be absorbed. Dissolution rate of the drugs depend on the surface area of the particles and solubility. The surface area of the drugs could be tuned by controlling the drug particle size. It is well known that the bioavailability of the water insoluble drugs can be improved by reduction in their particle size[1–3].

In the pharmaceutical industry, a number of conventional particle size reduction techniques have been utilized such as crushing, grinding, milling, spray drying, freeze-drying. These conventional strategies offer several disadvantages such as thermal and chemical degradation of drug ingredients due to high temperatures, high energy requirements, large amounts of solvent use, solvent-disposal problems, solvent residues, and broad particle size distributions (PSDs). Moreover, the dissolution rate and the oral bioavailability of pharmaceuticals received from these strategies differed widely, time consuming methods with poor flow characteristics and other handling difficulties[1,4,5].

Supercritical fluid technologies have successfully addressed above mentioned issues and in particular, supercritical carbon dioxide (sc-CO_2) has emerged as the primary process platform, since it is economical, scalable, non- toxic and environmentally compatible. Supercritical fluid technologies include a number of sub-process classifications such as, Rapid Expansion of Supercritical Solution (RESS), Supercritical Anti Solvent (SAS), Particles from Gas Saturated Solutions (PGSS), Depressurization of an Expanded Liquid Organic Solution (DELOS), micro-emulsion etc[5–8]. Among these, RESS and PGSS are found to be the most effective non-solvent processes. In this manusscript, we report our findings of the production of pharmaceutical molecular clusters of most commonly used drug molecules such as ibuprofen and naproxen, via. RESS processing. These molecular clusters were first trapped in "dry ice" and subsequently solubilized in DI water. The characterizations of drop casted and dried formulations resulted in stable, viscous liquid films.

2 EXPERIMENTAL

We used modified RESS process (shown in Fig.1) described in detail in our earlier publications[9–12]. In a typical RESS experiment, we loaded the dissolution chamber with a specific quantity of the starting material (e.g. ibuprofen powder = 200 mg) and sealed tightly. Liquid carbon dioxide (purity 99.9%) was injected into the chamber through a syringe pump. Raw ibuprofen / naproxen powder is dissolved in carbon dioxide with high speed mixer under formulation pressure and temperature, 325 bar and 40 °C, respectively. The drug mollecular clusters are collected in dual stage sealed vacuum traps initially cooled to liquid nitrogen temeprature. These collection conditions resulted in the entrapment of the molecular clusters in "dry ice". The "dry ice" containing trapped nanoparticles was sublimated in deionized water without any surfactant.

These formulations were assessed for *In vitro* cancer cell viability investigations using the CellTiter 96 AQueous One Solution Cell Proliferation Assay kit (Promega, Madison, WI). HeLa (ATCC no. CCL-2) cells were cultured in DMEM (Sigma) supplemented with 10% FBS

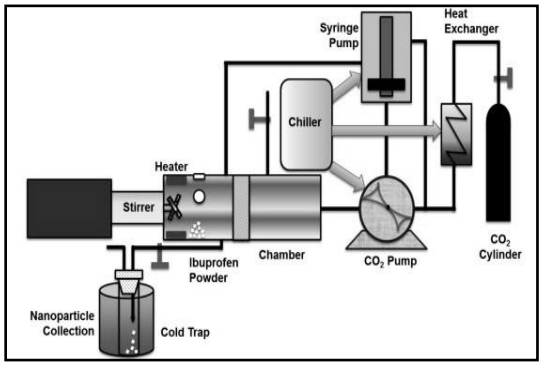

Fig. 1. Schematic diagram of modified RESS equipment.

(GE Healthcare Life Sciences, Logan, UT), 4 mM L-glutamine and 1% penicillin/streptomycin (all from Sigma) at 37 ºC and 5% CO_2. Once the cells reached ~ 95% confluence, they were split (using 0.25% trypsin-EDTA, Sigma) and plated at a density of 2×10^3 cells/well in 100 µL medium containing ibuprofen and naproxen formulations (water-solubilized or raw powders dissolved in dimethyl sulfoxide (DMSO)) at the desired concentrations in a 96-well plate (Falcon BD). 1% DMSO concentration is commonly used in cell studies including *in vitro* cell toxicity assays for testing NSAIDs such as ibuprofen[13]. After culturing for 72 h, the medium was replaced, and 20 µL MTS reagent was added and incubated for 3 h at 37 °C.

Following incubation the absorbance was measured at 490 nm using a microplate reader (Bio-Tek, Highland, WI), with a reference wavelength of 650 nm to subtract the background. Cells treated with vehicle alone (1% DMSO) were used as control, and wells with medium alone served as a blank. The percentage cell viability was determined from the ratio of the absorbance of the treated cells to the control cells. The results presented are an average of three independent experiments carried out on separate days.

The dispersion containing ibuprofen / naproxen dissolved in DI water were drop casted on silicon substrates and dried under ambient conditions for overnight. Optical micrographs of drop casted ibuprofen films were acquired on a Nikon Eclipse LY100POL microscope equipped with a Nikon Digital Sight DS-Fi2 camera. The optical images were collected using 50× objective with NA of 0.8. Surface morphology of the films was analyzed by a field emission scanning electron microscope (FE- SEM, Quanta FEG 450). The samples were examined at 10 kV accelerating voltage in low vacuum operation. The compositional identification was carried out using confocal Raman microscopy (WiTec alpha 300 confocal Raman microscope) using 532 nm excitation[14].

3 RESULTS AND DISCCUSION

HeLa cells were exposed to unprocessed and RESS processed ibuprofen and naproxen formulations containing 0.25 – 2.0 mM (shown in Fig. 2), respectively. After 72 hrs of incubation time , cell viability was assessed using a colorometric assay based on the reduction of MTS. These formulations resulted in inhibition of cancer cell proliferation in a dose-dependent manner. In the 0.25 – 0.75 mM dose range, limited loss of cell viability was observed, with exposure to RESS IPO resulting in a greater diminishment of cell viability compared to the other IPO formulations (MTS response was 87 ± 2 % and 77 ± 3% of the controls for 0.25 mM and 0.75 mM RESS IPO, respectively).

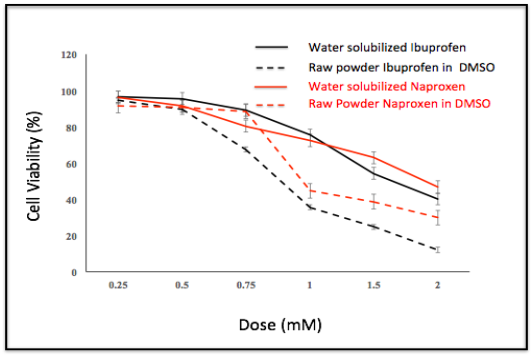

Fig. 2: In vitro cell viability results as a function of dosage level of unprocessed and RESS processed ibuprofen and naproxen formulations. RESS processed formulations of ibuprofen and naproxen (NSAIDs) exhibit similar cytotoxicity to the raw materials.

However, in the 1.0 – 2.0 mM dose range, significant loss of cell viability was observed for all formulations, with RESS IPO demonstrating the greatest potency. Exposure to 1 mM RESS IPO resulted in a dramatic decrease in viability of HeLa cells to approximately 16 ± 2% that of controls, compared to 75% and 36% cell viabilities following treatments with the same concentrations of IPO and raw IPO, respectively. Treatment with 2 mM RESS IPO decreased the cell viability even further, to 8 ± 2% that of controls (Fig. 2), which was approximately half of the viability observed following exposure to 2 mM IPO solution. Overall, our results show that ress IPO exhibits potent anti-proliferative effects in cancer cells. The lower cell viability observed for the raw NSAIDs compared to the water-solubilized materials can be attributed to the fact that the raw powders were necessarily solubilized in DMSO, owing to their poor solubility in water. DMSO is well known solvent for solubilizing poorly soluble molecules for biological assays. Discounting the solvent effects, the RESS processed water-solubilized NSAIDs exhibit similar cytotoxicity to the raw materials.

Optical micrographs of ibuprofen film dried from the aqueous formulations on a quartz substrate are shown in Fig. 3. A sharp pointed stainless steel needle is used to create a scratch on the film and compared the images of the same film location, as a function of time, Fig. 3a (after overnight ambient drying) and b (after 7 days).

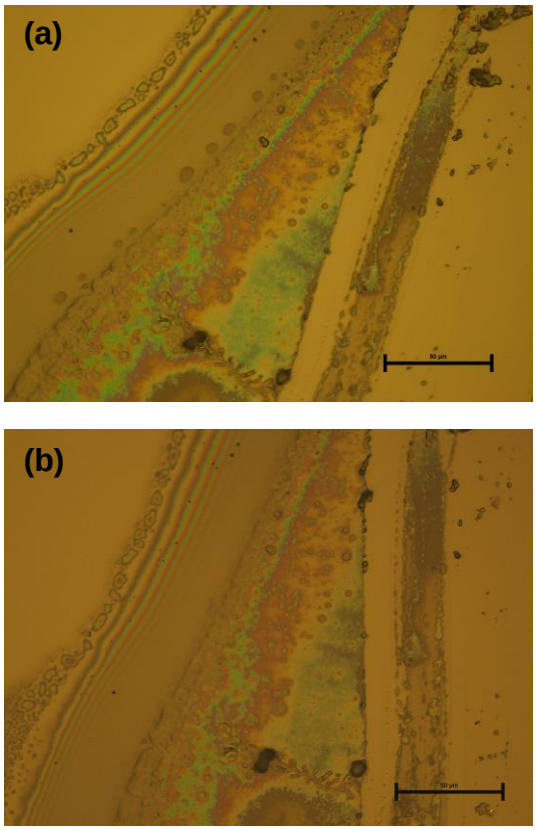

Fig. 3. Optical micrographs of ibuprofen thin films casted from aquesous dispersion (a) after overnight ambient drying and (b) after 7 days. The scale bar in the above optical images is 50 µm. The rpresence of noticable interference patterns are indicative of high qualiy films.

The presence of interference colors implies a high quality film and thesse are indicative of the thickness of the film. Over a period of 7 days, noticeable color (thickness) changes were observed at various locations in the film. This would imply that the film is viscous and spreading over the sub- strate surface like a liquid over this time period, thereby, getting thinner. It is fair to conclude that over a period of 7 days, the film surface has become more homogeneous, exhibiting "liquid-like" behavior[11,12,15].

The "liquid-like" behavior of these films were further investigated by using scanning electron miroscopy (SEM) shown in Figure 4. Inset of figure 4a shows the high resolution SEM micrograph of the same sample. Drop casted films are found to be homogeneous with slight autophobicity. These features are the indicative of high quality films.

Figure 5 shows the Raman spectra of unprocessed, RESS processed ibuprofen and naproxen, respectively. The bands in unprocessed and processed ibuprofen nanoparticles at 1570 and 1630 cm^{-1} correspond to the fingerprint region of the crystalline ibuprofen and naproxen

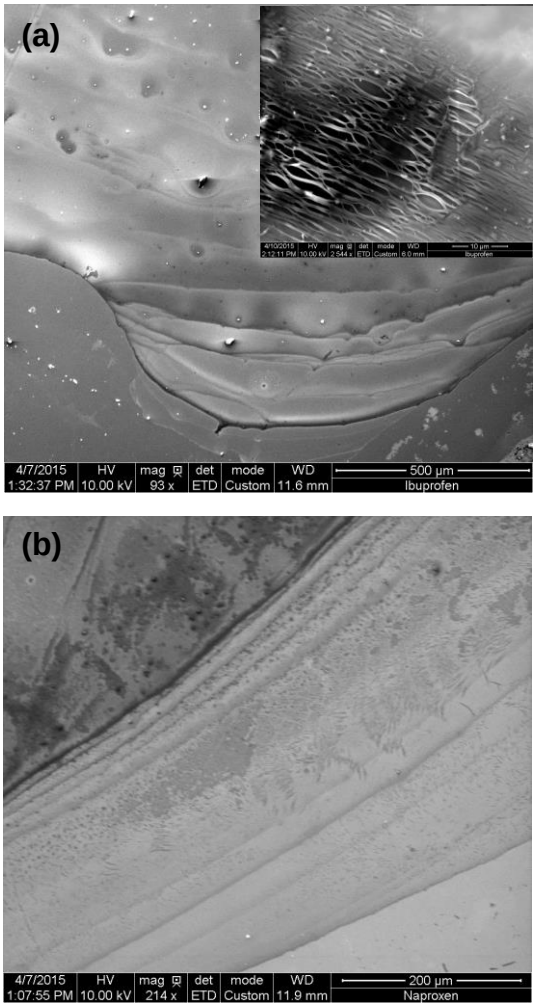

Fig. 4. SEM micrograph of drop casted (a) ibuprofen and (b) naproxen thin film over a period of 7 days. Inset of Figure 4a shows high resolution SEM micrograph of the same ibuprofen thin film showed a uniform, featureless film.

molecules, respectiely [16,17]. These specific bands located at a range of 750 to 900 and 900 to 1500 cm−1 are asigned due to C=O, aromatic C-H stretch and $-CH_2$, $-CH_3$ stretching, respectively [18,19].

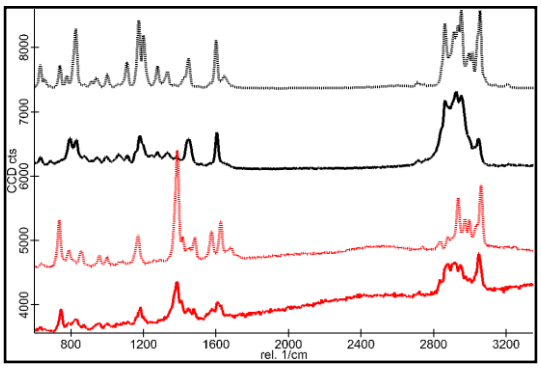

Fig. 5. Confocal Raman single spectra collected for unprocessed ibuprofen (black dotted line), RESS processed ibuprofen (black solid line), unprocessed naproxen (red dotted line) and RESS processed naproxen film (red solid line), respectively.

4 CONCLUSIONS

We report a method of production of pharmacutical molecular clusters of most commenly used phamacuticals such as ibuprofen and naproxen via RESS processing. Optical and SEM imaging of drop casted and ambient dried formulations showed high quality, "liquid like" viscous films with slight auto-phobic tendency. Our cell viability studies demonstrate that the water-solubilized ibuprofen and naproxen exhibit similar cytotoxicity to the raw materials. Confocal Raman characterizations confirmed that the molecular clusters of ibuprofen and naproxen retained their chemical identity after RESS processing.

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