



King's Research Portal

DOI:

[10.1016/j.ijpharm.2016.09.079](https://doi.org/10.1016/j.ijpharm.2016.09.079)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Goodwin, D. J., Martini, L. G., & Lawrence, M. J. (2016). Production of nabumetone nanoparticles: effect of molecular weight, concentration and nature of cellulose ether stabiliser. *INTERNATIONAL JOURNAL OF PHARMACEUTICS*, 514(2), 428–444. <https://doi.org/10.1016/j.ijpharm.2016.09.079>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Accepted Manuscript

Title: Production of nabumetone nanoparticles: effect of molecular weight, concentration and nature of cellulose ether stabiliser

Author: D.J. Goodwin L.G. Martini M.J. Lawrence



PII: S0378-5173(16)30925-5
DOI: <http://dx.doi.org/doi:10.1016/j.ijpharm.2016.09.079>
Reference: IJP 16121

To appear in: *International Journal of Pharmaceutics*

Received date: 19-6-2016
Revised date: 27-9-2016
Accepted date: 29-9-2016

Please cite this article as: Goodwin, D.J., Martini, L.G., Lawrence, M.J., Production of nabumetone nanoparticles: effect of molecular weight, concentration and nature of cellulose ether stabiliser. *International Journal of Pharmaceutics* <http://dx.doi.org/10.1016/j.ijpharm.2016.09.079>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Production of nabumetone nanoparticles: effect of molecular weight, concentration and nature of cellulose ether stabiliser

Goodwin DJ^{a,b} Martini LG^c, Lawrence MJ^a

^a*Institute of Pharmaceutical Science, King's College London, 150 Stamford Street, London, SE1 9NH*

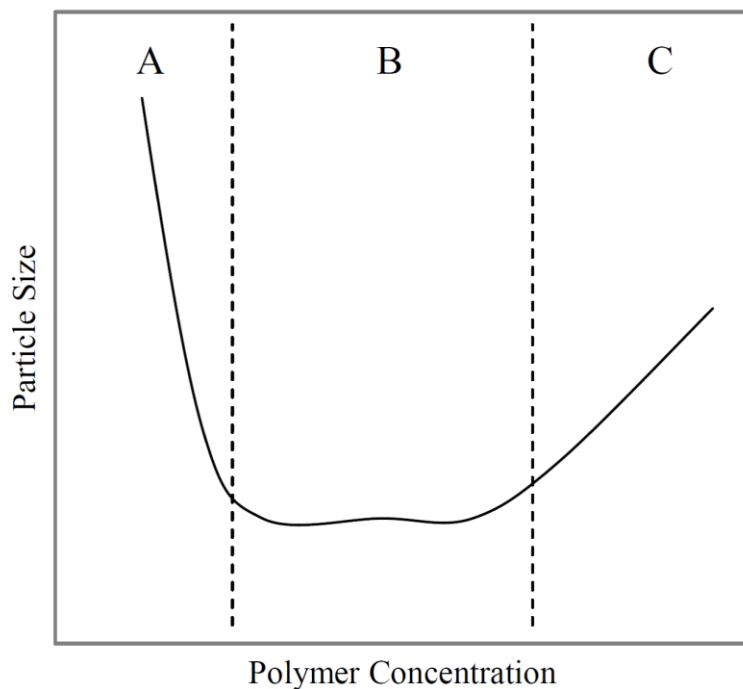
^b*GlaxoSmithKline, New Frontiers Science Park, Harlow, Essex CM19 5AW*

^c*GlaxoSmithKline, Park Road, Ware, Hertfordshire SG12 0DP*

^bcurrent address: GlaxoSmithKline, New Frontiers Science Park, Harlow, Essex CM19 5AW

^ccurrent address: Roche Products Limited, 6 Falcon Way, Shire Park, Welwyn Garden City, Hertfordshire AL7 1TW

Graphical abstract



Abstract

The ability of a range of hydrophilic nonionic cellulose ethers (CEs) (namely methylhydroxyethylcellulose, hydroxypropylmethylcellulose, ethylhydroxyethylcellulose, hydroxyethylcellulose and hydroxypropylcellulose) to prepare stable nabumetone nanoparticles (< 1000 nm, as measured by laser diffraction) using wet-bead milling has been investigated. Due to the limited range of CE molecular weights commercially available, the CEs were degraded using ultrasonication for varying lengths of time to yield CEs of lower molecular weight. Of the CEs tested, only hydroxyethylcellulose was found not to stabilise the production of nabumetone nanoparticles at any of the molecular weights tested, namely viscosity average molecular weights (M_v) in the range of 236 to 33 Kg/mol. All other CEs successfully stabilised nabumetone nanoparticles, with the lower molecular weight/viscosity polymers within a series being more likely to result in nanoparticle production than their higher molecular weight counterparts. Unfortunately due to the nature of the ultrasonication process, it was not possible to compare the size of nabumetone particles produced using polymers of identical M_v . There was, however, enough similarity in the M_v of the various polymers to draw the general conclusion that there was no strong correlation between the M_v of the various polymers and their ability to produce nanoparticles. For example hydroxypropylcellulose of 112.2 Kg/mol or less successfully produced nanoparticles while only ethylhydroxyethylcellulose and hydroxypropylmethyl polymers of 52 and 38.8 Kg/mol or less produced nanoparticles. These results suggest that polymer molecular weight is not the only determinant of nanoparticle production and that structure of the polymer is at least as important as its molecular weight. In particular the hydrophobic nature of the CE was thought to be an important factor in the production of nabumetone nanoparticles: the more hydrophobic the polymer, the stronger its interaction with nabumetone and the greater its ability to produce nanoparticles. In this context HPC was the most hydrophobic polymer and HEC the least hydrophobic.

Key Words: cellulose ethers, nabumetone, nanoparticle, wet-bead milling, molecular weight

Introduction

Poor water-solubility of a potential new drug is a major challenge when formulating it for use as medicine by patients. This is because in order for an orally administered drug to be absorbed, it must be dissolved in the gastrointestinal fluid at its site of absorption. If a drug has no or extremely low solubility in the gastrointestinal fluid, i.e. it is very hydrophobic, it is unlikely to be absorbed to sufficient extent to allow it to exert its therapeutic effect and will never reach the market and therefore the patient. While the poor aqueous solubility of drugs

has always been a challenge to formulators, the number of potential drugs that exhibit very poor water-solubility has increased significantly over recent years. This increase is widely attributed to the use of combinatorial chemistry and high throughput screening leading to new chemical entities with high molecular weight and increasing lipophilicity and therefore decreasing aqueous solubility. Indeed, it is estimated that, depending upon therapeutic area, up to 80-90% of new drugs currently under development exhibit poor water solubility (Thayer, 2010).

Many approaches have been explored to increase the rate of solution and the aqueous solubility of drugs, including salt formation, use of co-solvent, complexation with cyclodextrins, solubilisation in micelles and microemulsions amongst other things (Lawrence and Rees, 2012; Kalepu et al., 2015; Loh et al., 2015). One approach to improving drug dissolution rate and aqueous solubility that has recently attracted a lot of attention is the preparation of drug particles in the nanometre size range (typically between 10 to 1000 nm) – this then yielding particles with a high surface area-to-volume ratio and resulting in an increased dissolution rate and therefore potentially an improved *in vivo* performance of the poorly soluble drug (Loh et al., 2015; Li et al., 2016). Such drug nanoparticles can be produced by wet bead milling, using ceramic beads of millimeter diameter, crystalline drug in the presence of an aqueous solution of a surfactant and/or polymer to aid stability. Compared to other approaches to producing drug-containing nanoparticles for drug delivery, these nanoparticles have a number of advantageous properties. For example, the nanoparticles possess an exceptionally high drug loading, as the particle core is composed of pure drug material, with the stabilising surfactant and/or polymer comprising only a very low amount of the composite particle (Peltonen and Hirvonen, 2010). The ‘crystalline’ nanoparticles exhibit a high degree of stability, while Ostwald ripening of the nanoparticles can be reduced if the drug nanoparticles are relatively monodisperse and exhibit a very low aqueous solubility (Verma et al., 2010). It is possible to use the nanoparticles as is, i.e., in the form of a nano-suspension or to produce ‘dry’ nanoparticles that can be compressed into tablets or used as powder by freeze drying the suspension (Merisko-Liversidge et al., 2003), possibly in combination with a carrier such as mannitol. Indeed most of the crystalline nanoparticle products that have reached the market so far are produced by wet-bead milling (Van Eefenbrugh et al., 2008; Junghaus and Müller, 2008).

Despite this interest to date, however, no one single polymer or surfactant has been found suitable for stabilising all drugs, as different drugs have been found to require different stabilisers (Li et al., 2016). Furthermore, as no understanding exists on the interaction(s) between stabilizers and drugs, the 'best' stabiliser for a particular drug has to be determined on a case-by-case basis (Li et al., 2016). As part of a study aimed at better understanding of the relationship between stabiliser and drug, we have determined the effect of the molecular weight of polymer on the production of nanoparticles, their size and where appropriate, stability. To the authors knowledge this is the first time such a systematic study has been performed on the nature of the polymer used to stabilize drug nanosuspensions prepared by wet bead milling. Here, the poorly water soluble drug, nabumetone (Figure 1; aqueous solubility of 0.0047 mg/mL) was selected for study as previous studies have suggested that polymer molecular weight may be an important variable.

A range of structurally-related, commercially available nonionic cellulose ethers (CEs), namely methylhydroxyethylcellulose (MHEC), hydroxypropylmethyl cellulose (HPMC), ethylhydroxyethylcellulose (EHEC), hydroxyethylcellulose (HEC), and especially hydroxypropylcellulose (HPC) (Table 1) were examined for their ability to stabilise nabumetone nanoparticles prepared by wet bead milling. However, due to the limited range of CE molecular weights available commercially, ultrasonication was used to produce, using the methodology of Goodwin et al. (2011), CEs of varying molecular weights. Ultrasonication was selected as the method of CE degradation in the present study as it possess a number of important advantages over other available methodologies, including the fact that the polymer breaks towards the middle of the chain with no side reactions occurring and no monomers being formed during degradation (Kulicke et al, 2005).

Figure 1.

Table 1.

Materials and Methods

Materials

Nabumetone (Batch number 00N60141) was supplied by GlaxoSmithKline (Harlow, UK). Cellulose ethers (CEs) were used as received from the manufacturer with no corrections being made for moisture content. When available, pharmaceutical grade CE was purchased. The structure of the CEs was typically confirmed using a combination of UV, IR and NMR spectroscopy. Hydroxypropylcellulose (HPC(-EF); Klucel, nominal (M_w) molecular weight

80,000 Kg/mol, molar degree of substitution (ms) = 3.9, degree of substitution (ds) is not stated) and hydroxyethylcellulose (HEC; Natrosol 250G, nominal (M_n) molecular weight 300,000 Kg/mol, ms = 2.5, ds = 1.5) were obtained from Hercules (Wilmington, USA), hydroxypropylmethylcellulose (HPMC; Methocel(-4EM), nominal (M_n) molecular weight 86,000 Kg/mol, ms and ds not stated) from Colorcon (Dartford, UK), methylhydroxyethylcellulose (MHEC; Tylose MH50G4, nominal molecular weight not stated, ms and ds not stated) from Clariant (Wiesbaden, Germany) and ethylhydroxyethylcellulose (EHEC; Bermocoll E230G, nominal molecular weight (method weight not stated) 85,000 Kg/mol ms = 2.0 (molar degree of hydroxyethyl substitution), ds = 0.9 (degree of ethoxy substitution)). The quoted molecular weights of polymers are consequently viscosity average molecular weights (M_v). Table 2 gives the measured viscosity and viscosity (M_v) average molecular weights of the CE polymers used in the present study. Note that all polymer molecular weights quoted in this study are viscosity (M_v) average molecular weights. Aqueous stock solutions of the CEs were prepared by weighing the required mass of CE and adding about three quarters of the required volume of double-distilled water with constant stirring. After stirring for 19 h, the CE solution made up to final volume using double-distilled water. The resulting CE solution stored in a refrigerator (4 °C) for 24 h prior to its use. Yttrium zirconia (YTZ[®]) beads of diameter 0.44 mm (0.35 – 0.5 mm range) were obtained from the Nikkato Corp. (Tokyo, Japan). Spectroscopically-pure, double distilled water was used throughout the study.

Ultrasonication of Cellulose Ether Solutions

When the effect of polymer molecular weight on nanoparticle production was investigated, 1.5 wt% of CEs in aqueous solution were ultrasonically degraded. Although in the case of HPC-EF, degradation was also performed at 4 wt% of the CE in order to achieve solutions containing higher HPC concentrations). The method of ultrasonic degradation used was that of Goodwin et al (2011). In brief, the aqueous CE solution was placed in a screw-top glass jar within a jacketed bath containing water circulating at 2 °C. Ultrasonication of the CE solution was performed using a Soniprobe model 7535 A (Lucas Dawe Ultrasonics, UK) fitted with a tapered microtip operating at 15% of its maximum output (150 W). The CE solutions were ultrasonicated for either 1, 4, 8, 16 or 24 h (Table 2), with 24 h being the longest degradation time used as Goodwin et al. (2011) had previously established that the reduction in molecular weight of the CE polymer had plateaued off by this time. The molecular weight of the resulting sonicated polymer was determined as described below. After measuring the viscosity and viscosity (M_v) molecular weight, the polymer solutions were used without further manipulation.

Table 2.

Capillary Viscometry

All CE polymers used were characterized by determining their viscosity average molecular weight (M_v) using capillary viscosity on CE solutions that were of a concentration to ensure that the viscometric measurements were performed below C^* (defined as $1/[\eta]$, where $[\eta]$ is the intrinsic viscosity of the polymer). In all cases the measurements were performed in triplicate using a Ubbelohde (suspended level) viscometer immersed in a precision water bath at either 20 ± 0.1 °C for HPMC and 25 ± 0.1 °C for all other CE polymers (transparent thermostat CT 1650, Schott-Geräte, Hofheim, Germany) and connected to a Viscosity Measurement Unit AVS 350 (Schott-Geräte, Hofheim, Germany) and a ViscoDoser AVS 20 piston burette to allow the automatic dilution of the polymer solution. The intercept of separate Huggins and Kramer extrapolations of the measured flow times yielded the intrinsic viscosity $[\eta]$ and from this the molecular weight of the CE was determined by application of the Mark-Houwink equation when the necessary parameters were available (Goodwin et al. 2011).

Steric Exclusion Chromatography

Steric exclusion chromatography (SEC) was carried out on selected undegraded and lyophilized degraded HPC samples to obtain both a number-average (M_n) and weight-average (M_w) and to give an indication of polydispersity (P_d) of the sample ($P_d = M_w/M_n$) by Rapra Technology (Shrewsbury, UK). Degraded CE solution were lyophilized at -57 °C using a Heto PowerDry LL3000 (Thermo Electron Co., Bath, UK). A THF solution of each HPC sample of interest was prepared by adding THF (10 mL) to sample (20 mg) and leaving until dissolved (typically < 12 h) after which time the solution was warmed to 50 °C for 30 min and allowed to cool, after which it was filtered through a 0.2 μm polyamide membrane filter prior to SEC. SEC was performed using a PLgel guard column plus two mixed bed-B, 30 cm, 10 μm columns (Polymer Laboratories Ltd, Shropshire, UK) at 30 °C with THF as solvent at a nominal flow rate of 1.0 mL/min. Detection was based on measurement of the refractive index, with differential pressure and light scattering. The data were collected and analysed using Viscotek Trisec 2000 and Trisec 3.0 software. Calibration of the polymer molecular weight was achieved using polystyrene calibrants.

Preparation of Nabumetone Nanosuspensions Using Wet Bead Milling

Prior to their use, all polymer solutions were stored in a refrigerator at 4 °C for at least 24 h. Crude suspensions of nabumetone in the hydrophilic CE polymer solution were prepared prior to milling by the gradual addition with constant (magnetic) stirring of 5 g of nabumetone into 20 g of polymer solution. The crude nabumetone suspension thus prepared was left to stirring overnight prior to wet bead milling. For the purpose of the present study, the polymer concentration quoted is that of the CE polymer solution used to form the crude nabumetone suspension prior to milling and not the slightly lower final concentration of CE polymer in the crude nabumetone suspension. Wet bead milling of the crude nabumetone suspensions was carried out using a Retsch MM200 mixer mill (Glen Creston, Stanmore, UK). The milling jars (volume 25 cm³ with a fill capacity of 20 cm³) were made from Nylube, food grade nylon (Nylacast, UK). The milling conditions used in the present study were those developed for 'optimal' milling conditions by Sepassi et al. (2007). In brief, 10 cm³ of the crude nabumetone suspension was added to a milling jar containing 10 cm³ of 0.44 mm YTZ[®] beads and the crude suspension milled for 6 hours at a speed setting of 30 Hz. The particle size of the milled suspension was measured every hour by removing 0.05 mL for analysis by laser diffraction. After 6 hours milling, the YTZ[®] beads were removed using a 60 mesh sieve (0.25 mm nominal aperture) and the resulting nabumetone nanosuspension collected and stored in sealed vials for further analysis. The 6 hour data were selected for the end point of the milling as preliminary studies using 1.5 wt% hydrophilic CE polymer of varying molecular weight suggested that the size reduction was complete by this time point (Goodwin 2008). The stability of the nabumetone nanoparticles was determined by measuring their size when stored at ambient suspended in excess CE polymer in low light conditions (Valero and Costa, 2003) using laser diffraction and photon correlation spectroscopy 6, 9 and 12 months after preparation.

Physical Characterisation of Nanoparticles

Laser Diffraction Particle Size Analysis

A Malvern 2600 series laser diffractometer (Malvern Instruments, Malvern, UK) fitted with a Fourier transform lens providing a 63 mm focal length from a 2 mW He-Ne laser light source of wavelength 633 nm was used to size the nabumetone nanoparticles in suspension. Measurements were conducted at 25°C. Approximately 15 mL of water was added into a Malvern PS1 "Stirred Cell" sample holder providing a beam path length of 14.3 mm. After taking a background light scattering reading, the suspension was added drop-wise to the stirred cell until an acceptable obscuration reading (between 0.1 – 0.3) was achieved. Typically a 0.002

wt% solution of drug, corresponding to an obscuration value of approximately 0.2, was used. The size of the nanoparticles obtained from the laser diffraction measurements are recorded as the volume (or mass) moment mean ($D[4,3]$). The volume moment mean was selected for use with this study as it is sensitive to the presence of large particles in the sample (Rawle, 2002) which, even if they were small in terms of number, would make a up a considerable proportion in terms of mass. Particle size measurements on each suspension sample were performed in triplicate and were found to exhibit good repeatability, usually around $\pm 3 - 5 \%$.

Photon Correlation Spectroscopy

Photon correlation spectroscopy (PCS) measurements were performed using a Brookhaven ZetaPlus Particle Sizer v2.29 (Brookhaven Instruments Corp, UK) with a 677 nm He-Ne laser light source. Measurements were conducted in water at 20°C and the fluctuation of the light scattering from the nabumetone particles were measured at 90°. The average particle diameter was calculated over an average of 10 measurements each of 30 sec duration and was found to be independent of drug concentration in the range 0.0015-0.005 wt%. Routinely, three drops (~0.0075 mL) were taken from the suspension and added to 3 mL of water in a disposable clear 4-sided fluorescence cuvette for size analysis, giving a final concentration of 0.0025 wt%. A solvent viscosity of 0.89 cP was used as it assumed that the considerable dilution of the original suspension required to enable PCS size measurements to be made would mean that the dispersion would possess a viscosity very nearly approaching that of water (Fleer et al., 1993). Particle size measurements on each sample were performed in triplicate and were found to exhibit good repeatability.

Scanning Electron Microscopy

In order to obtain scanning electron microscopy (SEM) images of the nabumetone nanoparticles, a drop of the drug particle suspension was placed on the surface of a freshly cleaved piece of mica stuck to an SEM stub and allowed to air dry before being put in the electron microscope. The images were taken on an FEI Quanta 200F scanning electron microscope (FEI UK Limited, Cambridge, UK). The microscope was operated in low vacuum mode at a chamber pressure of 1.05 Torr (140 Pa) using an accelerating voltage of 5 kV and a small spot size to minimize specimen damage. The use of a low vacuum was essential, as Sepassi et al. (2007) found that nabumetone nanoparticles were prone to melt (melting point 78-83°C) under standard vacuum settings. Each nanosuspension sample was examined using SEM on one occasion.

Molecular Weight Studies of Adsorption

An investigation into the molecular weight dependence of polymer adsorption onto nabumetone particles was undertaken by wet bead milling for 2, 4 and 6 hours and characterising the molecular weight of the unadsorbed polymer using SEC. Three batches of crude suspension were prepared containing 20 wt% nabumetone and 1.5 wt% HPC-EF, either undegraded or ultrasonically degraded for either 4 or 24 hours. The crude nabumetone suspensions were milled for 2, 4 or 6 hours after which the resulting suspension was immediately sieved to remove the milling media and centrifuged to separate the supernatant containing the unabsorbed HPC polymer. The polymer supernatant was lyophilised (at $-57\text{ }^{\circ}\text{C}$ using a Heto PowerDry LL3000 (Thermo Electron Co., Bath, UK) freeze dryer) and its molecular weight analyzed by SEC to obtain both a number-average (M_n) and weight-average molecular weight (M_w) and to give an indication of the polydispersity (P_d) of the sample ($P_d = M_w/M_n$).

Results and Discussion

Nabumetone Nanoparticle Production: Effect of Polymer Type and Molecular Weight

The effect on the production of nabumetone nanoparticles, of the type and molecular weight of the five nonionic CEs of interest, was examined by milling nabumetone for 6 hours in the presence of a 1.5 wt% undegraded and ultrasonically degraded polymer solutions (Table 2). Significantly, it did not prove possible to mill nabumetone in the presence of HEC using any of the molecular weights available (Table 2) as the suspension solidified during milling suggesting that this particular polymer and drug combination was not suitable for nanoparticle production. In contrast, however, it was possible to obtain nanoparticles and micron-sized particles when milling in the presence of HPMC, MHEC, EHEC and HPC of varying molecular weight (Table 2).

Figure 2 shows the variation in particle size with milling time when HPMC, MHEC or EHEC was used as stabilizing polymer while Figure 3 shows the data obtained for HPC. Note that the data reported in Figure 2 are the mean of triplicate laser diffraction measurements on one sample (i.e. $n = 1$) of each system tested and reports no standard deviation, while the data in Figure 3 reports the mean and standard deviation of 3 replicate measurements on 3 individually milled samples. From the data shown in Figures 2 and 3, there is clear relationship between CE molecular weight and ability to form nabumetone nanoparticles, in that the lower molecular weight CE polymers within a series favour nanoparticle production, while

the highest molecular weight polymers are more likely to reach a minimum particle size that is greater than that achieved using their lower molecular weight counterpart.

Figure 2.

Figure 3.

In addition, Figures 2 and 3 show a definite trend within each series of CE polymer, of the systems containing the higher molecular weight polymer taking longer to mill to achieve a minimum size of nabumetone. The exponential decrease of the particle size with milling time seen in Figures 2 and 3 mirrors well that reported by other many other researchers, including ourselves (Sepassi et al., 2007). In the cases where a similarity in particle size was measured particle, it is suggested that particle breakage was the dominant mechanism of size reduction and that aggregation of the particles occurred at a smaller rate than particle breakage.

Although the rheological properties of the CE polymers were not examined in the present study, it is acknowledged that the more viscous, higher molecular weight CE's were likely to result in a dampening effect which will be expected to affect particle size reduction (Mende et al., 2003; Knieke et al., 2010). It has been proposed that an increased viscosity of the milling suspension is associated with a reduction in the forces of impaction in the mill, via a cushioning effect, resulting in a less efficient size reduction (Denison, 1990; Parrott, 1974). This hypothesis is in agreement with simulations of the motion of balls in a vibration mill performed by Yokoyama et al (1996) in which an increase in the viscosity of the surrounding medium caused a radical drop in the intensity of ball collisions.

Indeed it was expected that the lower molecular weight polymers would be more efficient at diffusing to, and coating, the freshly created 'bare' drug surfaces exposed during particle size reduction and thereby allow particle size reduction to proceed. It should be noted, however, that at 'equilibrium, it would be expected that the higher molecular weight polymers would replace the lower molecular polymers which initially coat, and thereafter rapidly desorb from the surface of the nanoparticles. The higher molecular weight polymers tend to remain 'stuck' on the surface due to their possession of a higher number of attachment points (Radeva, 2002). To illustrate this effect the change in the molecular weight and mo-

lecular weight distribution of the unadsorbed (and by implication the adsorbed) HPC remaining in the supernatant was determined after varying periods of wet bead milling with nabumetone in the presence of undegraded and ultrasonically degraded polymer (Table 3).

Table 3.

As can be seen from Table 3, the measured molecular weight of the HPC polymer remaining in solution, i.e. present in the supernatant, decreases with time of milling with nabumetone. This reduction is particularly noticeable when the polymer is HPC-EF UnD, where the M_w approximately halves from 110 to 66.5 Kg/mol over the 6 hours of milling, while the M_n decreases by almost a quarter from 40.3 to 32.1 Kg/mol. A similar, but less pronounced effect is also seen with HPC-EP USD4h and HPC-EF USD 24 h. If there was no preference in terms of molecular weight absorption onto the nabumetone then the M_w and M_n (and P_d) would remain unchanged with time of milling. Instead, the results obtained in the present study suggest that the higher molecular weight polymer is being preferentially absorbed, over time, onto the nabumetone. A similar observation has been reported by Sepassi-Ashnati (2003) when milling halofantrine with polyvinylpyrrolidone of differing molecular weights.

It is anticipated that, during milling, the drug crystal will fracture to expose the weakest attachment face, such that the properties of this weakest attachment face will dominate the milled state (York et al., 1998). Indeed, the more rapidly the drug crystal surface exposed during wet bead milling is coated with polymer, the less likely the fractured drug particles are to aggregate. When investigating nanoparticle formation using high pressure homogenisation, Müller and Jacobs (2002) proposed that the stabilisers are required to diffuse to the freshly created 'bare' drug surfaces during size reduction and that the rate of diffusion is dependent upon both stabiliser molecular weight and the viscosity of the solvent. While it is acknowledged that the mechanism by which high pressure homogenisation reduces particle size is not the same as that occurring by impaction in wet bead milling, the principle of stabilisation is comparable.

The fact that smaller polymer molecules are able to diffuse and adsorb more rapidly from solution (Morrison and Ross, 2002) and are more easily able to adopt a suitable conformation for adsorption may also facilitate the faster production of nanoparticles during wet bead milling since a greater proportion of particles that are fractured are covered in polymer before encountering another drug particle thereby inhibiting aggregation. The reduction in

the milling time required to produce nabumetone nanoparticles seen using lower molecular weight polymers has important implications. The most obvious of these is the economic advantage gained from the fact that less energy and time are required for nanoparticle production, especially when considering that the cost of a new technology is becoming increasingly important (Müller et al., 2001).

Despite the use of yttrium zirconia beads as milling media because of their reduced propensity for shedding and therefore containing the nanosuspension (Ruddy and Roberts, 1998), a concern remains that the milling media may contaminate the product (Jacobs et al., 2001; Liversidge et al., 1992). Any reduction in milling time can only further act to diminish this effect and further assure the quality of the product. Microbiological contamination can also arise during wet bead milling due to the moderate temperature increase encountered in the milling jars and the fact that the dispersion media can act as a nutrition source for bacteria (Hu et al., 2004). Again, a reduced milling time may serve to limit this problem.

While it was not possible in the present study to compare directly the size of nabumetone particles produced by wet bead milling using CE polymers of exactly the same molecular weight, there was enough similarity in polymer molecular weight to draw some general conclusions. Firstly, there was no strong correlation between polymer molecular weight and ability to produce nanoparticles. For example, it was not possible to obtain either nano- or even micron-sized particles using HEC of any of the molecular weights examined (Table 2). It was, however, possible to prepare nanoparticles using HPMC of 38.8 Kg/mol molecular weight, EHEC of 52 Kg/mol molecular weight or less and HPC of 112.2 Kg/mol molecular weight or less. (Note that it was not possible to determine the (viscosity average) molecular weight of MHEC that produced nanoparticles as the Mark-Houwink parameter was not available to convert measured viscosity to polymer molecular weight.) Furthermore, micron-sized nabumetone particles were formed using the lowest molecular weight HPMC polymer, while the highest molecular weight HPC used resulted in nano-sized particles. These results suggest that polymer molecular weight is not the only determinant of the final particle size and that structure and composition of the polymer are at least as important as molecular weight.

Due to the unionised nature of nabumetone and the nonionic CE polymers used in the present study, it is most likely that the predominant mechanism of interaction between the CE polymers and nabumetone is non-specific, and most probably hydrophobic, in nature (Duro et al., 1999; Lothead, 1992). As a consequence, it is thought that the hydrophobic nature of

the CE is an important factor in the production of nabumetone nanoparticles; the more hydrophobic the polymer, the stronger the interaction with the drug. In a study comparing the interfacial properties of three of the CEs used in the present study, namely HEC, HPMC and HPC, it was found that HPC was the most hydrophobic compound and HEC the least, with HPMC ranking intermediate (Daniels and Barta, 1994). The more hydrophilic nature of HEC may help to explain why it does not facilitate the production of nabumetone nanoparticles as it may be anticipated that the attraction between HEC and the aqueous solvent is greater than the interaction between HEC and drug, resulting in a reduced propensity for the HEC polymer to adsorb onto the surface of the drug. Note that while MHEC and EHEC were not included in the study by Daniels and Barta (1994), it appears from the results obtained in the present study, that the presence of the methyl and ethyl groups on the sugar units are sufficient to increase the hydrophobicity of the polymers and thus cause the polymers to adsorb on the drug surface and stabilise nabumetone nanoparticles.

Nabumetone Nanoparticle Production: Effect of HPC Molecular Weight and Concentration

As a consequence of the ability of the HPC polymers (over the whole molecular weight range available in the present study, namely 112, 89 or 57 Kg/mol) to produce nabumetone nanoparticles, it was decided to use the HPC polymers for further, more detailed studies. The effect of HPC molecular weight on the variation in particle size (as assessed by either laser diffraction or photon correlation spectroscopy) of nabumetone suspensions with polymer concentration was carried out using ultrasonically degraded polymer over a wide range of polymer concentrations, namely 0.5 - 4.0 wt% (Figure 4). Note that we have previously reported the difference in the particle size measured by laser diffraction and photon correlation spectroscopy (Sepassi et al., 2007). Note too that, regardless of HPC molecular weight, it was not possible to produce nabumetone nanoparticles using less than 0.5 wt% HPC. A similar requirement for a minimum amount of stabiliser to be present during wet bead milling has also been noted by others researchers including Liversidge and Cundy (1995).

Figure 4.

As can be seen at the lowest polymer concentrations studied, namely 0.5 - 2.0 wt%, the molecular weight of HPC had little effect on the particle size of the resulting nabumetone nanoparticles. Interestingly, this observation conflicts with the results reported in the earlier study of Sepassi et al., (2007) who found that the minimum concentration of the polymer, HPMC, required to form nabumetone nanoparticles during wet bead milling approximately

doubled from 0.63 to 1.25 wt% as the molecular weight of HPMC increased from 5.0 to 31.3 Kg/mol. A possible explanation for this difference in behaviour observed with HPC and HPMC stabilised nanoparticles is the much wider molecular weight range of HPMC used compared to HPC, i.e. ~ 6 fold as opposed to ~ 2 fold. The smaller molecular weight range available for HPC was partly a result of the decreased reduction in molecular weight obtained when degrading HPC and the limiting decrease in molecular weight of the polymer under the experimental conditions used (Goodwin et al., 2011). However, the advantage of using ultrasonically degraded polymer as compared with the commercially available grades at different molecular weights from different manufacturers as used by Sepassi et al. (2007), is that there is more confidence that any differences in adsorption noted between polymers is due solely to molecular weight effects rather than in substitution pattern or other manufacture to a manufacturer variations. The use of a wider molecular weight range of HPC may be expected to lead to a similar observation as seen by Sepassi et al. (2007).

The most noticeable effect of polymer molecular weight occurs when HPC concentrations of greater than 2.0 wt% were used, where a distinct relationship between polymer molecular weight and the maximum polymer concentration at which nanoparticles are produced, was observed. When using HPC of molecular weight 106 kg/mol, the particle size of nabumetone increased to almost 1 μm (as assessed by laser diffraction) at 2.5 wt% HPC. Whereas when using HPC of molecular weights of 89 of 57 kg/mol, the nabumetone particles only reached 1 μm (as assessed by laser diffraction) when the HPC concentrations were 3.5 and 4.0 wt% HPC, respectively. The reason why nanoparticles can be stabilized at higher concentrations when using with low molecular weight polymer is thought to be the result of the lower viscosity of the polymer solution allowing the milling beads to travel faster during milling, leading to a higher force of impaction on the nabumetone particles.

For industrial formulation purposes, the implication of this finding is that there is more flexibility to alter the polymer concentration when using a lower molecular weight polymer without affecting the size of the drug nanoparticles produced. This observation may be beneficially used, for example, to tailor the rheological properties of the milled nanosuspension to allow for further processing in instances where viscosity can influence the performance of such processes, such as spray drying or use as a binder for granulation.

Nabumetone Nanoparticle Production: Effect of HPC Molecular Weight and Concentration on Morphology

In order to further understand the size distribution of the nabumetone nanoparticles obtained from laser diffraction (and photon correlation spectroscopic) measurements as well as obtain shape and structural information, it is beneficial to visualise the nabumetone particles in the milled suspension (Shi et al., 2003). Examples of the electron scanning micrograph (SEM) images of nabumetone nanoparticles when milled for 6 hours with varying HPC concentration and molecular weight are shown in Figure 5.

Figure 5.

From the SEM images it appears that, at low HPC concentrations of 0.5 wt%, most large nabumetone particles tend to show cuboidal morphology, whereas at higher polymer concentrations of 1.5 and 3.0 wt%, the larger particles become more elongated. This effect may be the result of the viscosity of the dispersion medium wherein a lower viscosity results in a higher proportion of the particles being fragmented, whereas cleavage or abrasion is the predominant size reduction mechanism when the force of impact is reduced (Varinot et al., 1997) due to the increased viscosity of the dispersion. Previously, Merisko-Liversidge et al. (2003) have suggested that the morphology of the nanoparticles is dictated by the morphology of the starting material, the fracture plane of the drug crystal and the drug/stabilizer interactions, while Sepassi et al. (2007) proposed that nanoparticle morphology was dependent on the nature of the stabiliser. From the results of the present study, it appears polymer concentration has little impact on the morphology of the resulting nanoparticles.

The SEMs indicate that the nanoparticles are fairly polydisperse with respect to both size and shape especially at the extremes of the studied HPC concentration range, i.e. 0.5 and 3.0 wt%. It is clear from the SEMs that the nabumetone nanoparticles were smallest when intermediate HPC concentrations were used as well as when using lower molecular weight polymer; results which confirm the findings of the laser diffraction measurements. In addition, it appears from the SEM images, that when using 3.0 wt% HPC, the nabumetone particles appear to be large, while in contrast when using 0.5 wt% HPC the individual nanoparticles are relatively small although they may exhibit agglomeration or aggregation. This observation is particularly noticeable when using the high molecular weight, 106 Kg/mol, polymer (Figure 5a).

Nabumetone Nanoparticle Production: Effect of Milling Time on Size and Morphology

The SEMs of nabumetone particles obtained after wet bead milling in the presence of various molecular weights of 1.5 wt% HPC for 2, 4 and 6 hours are shown in Figure 6.

Figure 6.

Figure 6 shows that when wet bead milling nabumetone with 1.5 wt% HPC of molecular weight of 109 kg/mol, there is a large proportion of particles present with a particle size greater than 3 μm after 2 and 4 hours milling. After 6 hours milling the polydispersity of the sample had decreased such that the majority of particles were around 1 μm in size and possessed a columnar morphology. Decreasing the molecular weight of HPC to 89 kg/mol (Figure 6b) and 56 kg/mol (Figure 6c) eliminated the majority of these large particles at the shorter milling times of 2 h and 4 h, and yielded nanoparticles after 4 and 6 h that tended to be much more spherical in shape, although there were still some larger rod-shaped particles present, albeit in the sub-micron size range.

Nabumetone Nanoparticle Stability

The variation in nabumetone nanoparticle size, as assessed by laser diffraction, with storage time at room temperature in the presence of excess polymer is illustrated in Figure 7, using laser diffraction. From the particle size results shown it appears that the nabumetone particles were physically stable over the whole of the studied HPC concentration and molecular weight range, with no apparent increase in particle size over 12 months storage.

Figure 7.

Ostwald ripening is considered to be one of the main sources of (long-term) physical instability of drug nanoparticle suspensions (Grau et al, 2000; Jacobs et al, 2001; Müller and Keck, 2004; Müller et al, 2001). The phenomenon arises from the different saturation solubilities exhibited by a dispersion with a heterogeneous size distribution. The preferential dissolution of smaller particles leads to supersaturation of the solution and a subsequent recrystallisation onto the surface of the larger particles causing the larger particles to grow (Müller et al., 2001). One strategy to limit Ostwald ripening is to ensure that the drug nanoparticles exhibit as narrow a size polydispersity as possible (Grau et al., 2000; Jacobs et al., 2000). In this context, the SEM images of the nabumetone particles, clearly show polydispersity in size, particularly those prepared using the extremes of polymer concentration – although these particular nanosuspensions surprisingly exhibit no instability with respect to

particle size. In this context, the presence of the adsorbed polymer layer may have an additional role in providing a protective layer against recrystallisation and particle growth (Ziller and Ruprecht, 1991). Indeed it has long been known that polymers, including some CEs (Raghavan et al, 2001), inhibit the process of crystallisation by occupying the adsorption sites on a crystal lattice where drug molecules would attach (Raghavan et al, 2000; Simonelli et al, 1970; Ziller and Ruprecht, 1988). This inhibition of crystal growth (Lee et al, 2000) may explain the stable nature of these polymer coated nanoparticles.

Of course, the requirement for a system to remain stable over weeks or months is of less importance if a nanosuspension is dried (e.g. lyophilised or spray-dried) immediately after production and formulated into a solid dosage form (Merisko-Liversidge et al., 2003). Regardless, of the final form of the nanoparticles, however it is essential is that the nanoparticles remain, or more accurately retain the ability to redisperse into discrete nanoparticles *in vivo* and the presence of long term nanoparticle size stability may be a useful indicator of an adsorbed layer of sufficient thickness and stability for this to occur.

In order to summarise the effect of HPC concentration, molecular weight and the duration of milling time on the particle size of nabumetone suspensions, contour plots which include additional size analysis results after milling between 2 and 6 hours at different polymer concentrations are shown in Figure 8.

Figure 8.

Figure 8 clearly shows an increased region of nanoparticle formation (blue shaded area) when the lower molecular weight HPC is used, nanoparticles being formed more quickly and using a wider range of polymer concentrations. When the highest molecular weight polymer, the commercially available HPC(-EF) was used (Figure 8a) the region of nanoparticle formation is relatively narrow and there is a need to mill for longer periods to achieve a particle size (as assessed using laser diffraction) of less than 1 μm . These contour plots provide a useful guide for the formulator in determining which polymer concentration to use at a certain molecular weight. For example, from Figure 8 a polymer concentration of 1.5 wt% at a HPC M_v molecular weight of 57 kg/mol is clearly in the middle of the range where nabumetone nanoparticles are be formed and as such small variations in HPC concentration should not result in the formation of microparticles.

Using the data obtained for the effect of HPC concentration on the particle size profile obtained from the wet bead milling of nabumetone it is possible to divide the results into three parts, namely region A, B and C (Figure 9), with nanoparticles being formed by HPC concentrations in region B. Indeed it is considered that the profile illustrated in Figure 9 is general and can be applied to milling with a different polymer, e.g. HPMC (Sepassi et al., 2007). Although it should be noted that the concentration range over which nanoparticles are formed will vary with the nature of the drug and polymer studied (Liversidge et al., 1992; Sepassi et al., 2007).

Figure 9.

The increases in particle size seen when milling in the presence of low and high concentrations of polymer, i.e. concentrations in the regions denoted A or C are thought to be the result of different effects. In region A, the small amount of polymer present is considered to be insufficient to fully coat the drug particle in order to form a protective layer against aggregation/agglomeration. In addition, the presence of the relatively high drug loading required for wet bead milling, combined with low polymer concentrations in region A predisposes the nanoparticles to bridging flocculation, whereby an individual polymer molecule becomes attached to two or more particles causing them to flocculate (Lothead, 1992). The large particle size observed in polymer concentration region A is therefore the result of either agglomeration or aggregation of the nanoparticles and/or the occurrence of bridging flocculation.

The increase in particle size seen in the polymer concentration range in region C is frequently attributed to Ostwald ripening (Merisko-Liversidge et al., 2003). Although, in this context, the absence of any increase in nabumetone particle size over 12 months storage observed in the present study, suggests that Ostwald ripening might be less important than originally thought. Alternatively, Sato and Kohnosu, (1994) have suggested the particle growth may be due to the phenomenon of depletion flocculation, i.e. where non-adsorbing polymer destabilises dispersions due to an osmotic effect that occurs when polymer molecules are excluded from interparticle regions leading to an attraction force between particles and subsequent particle growth (Nashima and Furusawa, 1991). Another possible explanation is that the increased viscosity associated with increasing polymer concentration reduces the forces of impaction in the mill, via a cushioning effect, resulting in a less efficient size reduction (Denison, 1990; Parrott, 1974). This hypothesis is in agreement with simulations of the motion of balls in a vibration mill performed by Yokoyama et al (1996) in which an increase in the viscosity of the surrounding medium caused a radical drop in the intensity of

ball collisions. Indeed, it is not possible to mill using high concentrations of polymer due to high viscosity of the system. The growth of the particles may be due to one or all of the above mechanisms. However, regardless of the causative mechanism(s), the ultimate effect of the increase in particle size seen in Region C, is the loss of the increased surface area and reduction in bioavailability.

The size of nabumetone nanoparticles obtained when milling in the presence of HPC over the concentration range B remained fairly constant and is thought to be dependent upon the balance between the hardness of the drug, the energy input in the mill and the bead size, amongst other things. In fact the minimum particle size (as assessed by laser diffraction) of $\sim 0.75 \mu\text{m}$ (or $\sim 0.4 \mu\text{m}$ using photon correlation spectroscopy) achieved for nabumetone after 6 hours milling is relatively high compared to other studies using other drugs where sizes of 400 nm (Liversidge, 1991) or even 200 nm (Merisko-Liversidge et al., 2003) have been reported and suggests that nabumetone is particularly hard to mill to a small particle size. The low melting point of nabumetone could account for this result as Kondo et al. (1993) have reported that empirically, a drug with a high melting point is preferable for micronisation since it is easier to fracture into small particles and this may exacerbated during wet bead milling due to the increase in temperature that was observed during milling (Parrott, 1985).

It is worth noting, however, that it is not essential that the drug nanoparticles are as small as possible for every application since it depends on the therapeutic class and therefore the intended use of the drug in question (Müller et al., 2001). In cases where very fast dissolution is required, a size of 100-200 nm is sometimes quoted as the target particle size (Müller et al., 2001), Yamada et al. (1999) have observed moderate increases in dissolution of up to four-fold by reducing the particle size of a poorly water soluble model drug from $\sim 8 \mu\text{m}$ to $\sim 1 \mu\text{m}$. It is also reported that the saturation solubility of a compound is observed to increase as the particle size falls below 1-2 μm (Keck and Müller, 2006; Mosharraf and Nyström, 1995) which further increases the dissolution rate of a compound according to the Noyes-Whitney equation.

Conclusions

A range of hydrophilic nonionic CEs (methylhydroxyethylcellulose (MHEC), hydroxypropylmethyl cellulose (HPMC), ethylhydroxyethylcellulose (EHEC) and hydroxypropylcellulose (HPC)) of varying molecular weight (measured as a viscosity average molecular weights

(M_v)), have been found to prepare stable nabumetone nanoparticles (< 1000 nm, as measured by laser diffraction) using wet-bead milling. Only hydroxyethylcellulose (HEC) was found not to produce nabumetone nanoparticles at any of the molecular weights tested. Although molecular weight was important in that the lower the CE molecular weight the greater the likelihood of nanoparticle production than their higher molecular weight counterparts, the results suggest that polymer molecular weight is not the only determinant of nanoparticle production and that structure of the polymer is at least as important as its molecular weight. In particular the hydrophobic nature of the CE is an important structural factor in the production of nabumetone nanoparticles: the more hydrophobic the polymer, the stronger its interaction with nabumetone and the greater its ability to produce nanoparticles. In this context HPC was the most hydrophobic polymer and HEC the least hydrophobic.

The nabumetone nanoparticles produced using HPC of different molecular weights and concentrations demonstrated good long-term physical stability for up to at least one year. Both the concentration and molecular weight of the stabilising HPC affected whether nanoparticles of nabumetone could be produced. Using too low or too high a concentration of HPC polymer resulted in the formation of large particles of greater than 1 μm , a phenomenon which is thought to be due to agglomeration and a reduced size reduction, respectively. In contrast nabumetone nanoparticles could be produced when milling in the presence of intermediate concentrations of HPC. Furthermore, as the molecular weight of the HPC was decreased, the concentration over which nanoparticles could be formed was increased, while the milling time required for nanoparticle production was reduced. Although this general effect is found to occur for other CEs, the relevant CE concentrations and molecular weights are considered to be drug and polymer specific.

Acknowledgements

D.G. would like to thank the EPSRC, Impact Faraday and GlaxoSmithKline for the award of a studentship.

References

Daniels, R. and Barta, A. (1994). Pharmacopoeial cellulose ethers as oil-in-water emulsifiers I. Interfacial properties. *European Journal of Pharmaceutics* **40**: 128-133.

Dension, B. (1990) Bead milling - a practical guide. *Journal of the Oil Colour Chemists Association*. **6**: 256-261.

Duro, R., Souto, C., Gomez-Amoza, J.L., Martinez-Pacheco, R. and Concheiro, A. (1999). Interfacial adsorption of polymers and surfactants: Implications for the properties of disperse systems of pharmaceutical interest. *Drug Development and Industrial Pharmacy* **25**: 817-829.

Fleer, G.J., Cohen Stuart, M.A., Scheutjens, J.M.H.M., Cosgrove, T. and Vincent, B. (1993). *Polymers at Interfaces*. Chapman and Hall: London.

Goodwin, D.J. (2007) *Polymer-stabilised drug nanoparticles*. PhD Thesis London University.

Goodwin, D.J., Picout, D.R., Ross-Murphy, S.B., Holland, S.J., Martini, L.G., Lawrence, M.J. (2011). Ultrasonic degradation for molecular weight reduction of pharmaceutical cellulose ethers. *Carbohydrate Polymers* **83**: 843-851.

Goodwin D.J., Sepsis, S., King, S.M., Holland, S.J., Martini, L.G., Lawrence, M.J. (2013) Characterisation of polymer adsorption onto drug nanoparticles using depletion measurements and small-angle neutron scattering. *Molecular Pharmaceutics* **10**: 4146-4158.

Grau, M.J., Kayser, O. and Müller, R.H. (2000). Nanosuspensions of poorly soluble drugs - reproducibility of small scale production. *International Journal of Pharmaceutics*. **196**: 155-157.

Hu, J., Johnston, K.P. and Williams, R.O. (2004). Nanoparticle engineering processes for enhancing the dissolution rates of poorly water soluble drugs *Drug Development and Industrial Pharmacy* **30**: 233-245.

Jacobs, C., Kayser, O. and Müller, R.H. (2001). Production and characterisation of muco-adhesive nano suspensions for the formulation of buprivoquone. *International Journal of Pharmaceutics*. **214**: 3-7.

Junghaus, J.-U. and Müller, R.H. (2008). Nanocrystal technology, drug delivery and clinical applications. *International Journal of Nanomedicine*. **3**: 295–310.

Kalepu, S. and Nekkanti, V. (2015). Insoluble drug delivery strategies: review of recent advances and business prospects. *Acta Pharmaceutica Sinica B*. **5**: 442-453.

Keck, C.M. and Müller, R.H. (2006). Drug nanocrystal of poorly soluble drugs produced by high pressure homogenisation. *European Journal of Pharmaceutics and Biopharmaceutics*. **62**: 3-16.

Knieke, C., Steinborn, C., Romeis, S., Peukert, W., Breitung-Faes, S. and Kwade, A. (2010). Nanoparticle production with stirred-media mills: opportunities and limits. *Chemical Engineering Technology*. **33**: 1401-1411.

Kondo, N., Iwao, T., Masuda, H., Yamanouchi, K., Ishihara, Y., Yamada, N. et al (1993). Improved oral absorption of a poorly water-soluble drug, HO-221, by wet-bead milling producing particles in sub micron region. *Chemical and Pharmaceutical Bulletin*. **41**: 737-740.

Kulicke, W.M., Clasen, C. and Lohman, C. (2005). Characterisation of water-soluble cellulose derivatives in terms of the molar mass and particle size as well as their distribution. *Macromolecular Symposium*. **223**: 151-174.

Lawrence, M.J. and Rees, G.D. (2012) Microemulsion-based media as novel drug delivery systems. *Advanced Drug delivery Reviews*. **64**: 175-193S.

Lee, R.W., Shaw, J.M., McShane, J. and Wood, R.W. (2000) Particle size reduction. In: Liu, R., editor. *Water-insoluble drug formation*. Interpharm Press: Buffalo Grove: 455-492.

Liversidge, G.G. and Cundy, K.C. (1995) Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I Absolute oral bioavailability of nano crystalline danazol in beagle dogs. *International Journal of Pharmaceutics*. **125**: 91-97.

Liversidge, G.G., Cundy, K.C., Bishop, J.F. and Czekai, D.A. (1992). Surface modified drug nanoparticles. US Patent No. 5145684.

Lothead, R.Y. (1992). Water soluble polymers solution adsorption and interaction characteristics. *Cosmetics and Toiletries* **107**:131-156.

Loh, Z.H., Samanta, A.K. and Heng, P.W.S. (2015). Overview of milling techniques for improving the solubility of poorly water-soluble drugs. *Asian Journal of Pharmaceutical Science*. **10**: 255-274.

Mende, S., Stenger, F., Peukert, W. and Schwedes, J. (2003) Mechanical production and stabilization of submicron particles in stirred media mills. *Powder Technology*. **132**: 64-73.

Meng, L., Azad, M., Dave, R. and Bilgili E (2016). Nanomilling of drugs for bioavailability enhancement: a holistic formation-process perspective. *Pharmaceutics*. **8**: 1-35.

Merisko-Liversidge, E., Liversidge, G.G. and Cooper, E.R. (2003) Nanosizing: a formulation approach for poorly-water-soluble compounds. *European Journal of Pharmaceutics and Biopharmaceutics*. **18**: 113-120.

Morrison, I.D. and Ross, S. (2002). Colloidal Dispersions: Suspensions, Emulsions and Foams. John Wiley and Sons: New York.

Mosharraf, M. and Nyström, C (1995). The effect of particle-size and shape on the surface specific dissolution rate of micronised practically insoluble drugs. *International Journal of Pharmaceutics*. **122**: 35-47.

Müller, R.H. and Jacobs, C. (2002). Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long term stability. *International Journal of Pharmaceutics*. **237**: 151-161.

Müller, R.H. and Keck, C.M. (2004) Challenges and solutions for the delivery of biotech drugs - a review of drug nanocrystal technology and lipid nanoparticles. *Journal of Biotechnology*. **113**: 151-170.

Müller, R.H., Jacobs, C. and Kayser, O. (2001). Nanosuspensions as particulate drug formulations in therapy. Rationale development and what we can expect for the future. *Advanced Drug Delivery Reviews*. **47**: 3-19.

Nashima, T. and Furusawa, K. (1991). The rheological behaviour of a latex dispersion including a non-ionic polymer. *Colloids and Surfaces*. **55**: 149-161

Parrots, E.L. (1974) Milling of Pharmaceutical Solids. *Journal of Pharmaceutical Sciences*. **63**: 813-829.

Parrott, E. (1985) Milling. In: Lachman, L., Lieberman, H.A. and Kanig, J.L. editors. The Theory and Practice of Industrial Pharmacy. 3rd edition. Lee and Febiger: Philadelphia: 21-46.

Peltonen, L. and Hirvonen, J. (2010) Pharmaceutical nanocrystal by nano milling: critical process parameters, particle fracturing and stabilisation methods. *Journal of Pharmacy and Pharmacology* **62**: 1569-1579.

Raghavan, S.L., Trividic, A., Davis, A.F. and Hadgraft, J. (2000) Effect of cellulose polymers on supersaturation and in vitro membrane transport of hydrocortisone acetate. *International Journal of Pharmaceutics*. **193**: 231-237.

Raghavan, S.L., Trividic, A., Davis, A.F. and Hadgraft, J. (2001) Crystallisation of hydrocortisone acetate: influence of polymers. *International Journal of Pharmaceutics*. **212**: 213-221.
Ruddy, S.B. and Roberts, M.E. (1998). Nanoparticles containing the R(-) enantiomer of ibuprofen. US Patent No. 5718919.

Radeva, T. (2002) Adsorption of polymer at colloid surfaces. In Hubbard A, Editor Encyclopedia of Surface and Colloid Science. New York: Marcel Dekker p 547-557.

Sepassi-Ashtiani, S. (2003). *Polymer-Stabilised Drug Nanoparticles*. PhD Thesis. University of London.

Sepassi, S., Goodwin, D.J., Drake, A.F., et al., (2007). Effect of polymer molecular weight on the production of drug nanoparticles. *Journal of Pharmaceutical Sciences*. 96:2655-2666.

Sato, T. and Kohnosu, S. (1994). Effect of polyvinylpyrrolidone on the physical properties of titanium dioxide suspensions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. **88**: 197-205.

Shi, H.G., Farber, L., Michaels, J.N., Dickey, A., Thompson, K.C., Shelukar, S.D. et al., (2003). Characterisation of crystalline drug nanoparticles using atomic force microscopy and complimentary techniques. *Pharmaceutical Research*. **20**: 479-484.

Simonelli, A.P., Mehta, S.C. and Higuchi, W.I. (1970). Inhibition of sulfathiazole crystal growth by polyvinylpyrrolidone. *Journal of Pharmaceutical Science*. **59**: 633-638.

Thayer, A.M. (2010) Finding solutions. *Chemical and Engineering News*. 88: 13–18.

Valero, M. and Costa, S.M.B. (2003). Photodegradation of nabumetone in aqueous suspension. *Journal of Photochemistry and Photobiology A: Chemistry*. **157**: 93-101.

Van Eerdenbrugh, B., Vernabt, J., Martens, J.A., Van Den Mooter, G and Augustijns, P.(2010) Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. *International Journal of Pharmaceutics*. **364**: 64–75.

Variant, C., Hiltgun. S., Pons, M. and Dodds, J. (1997). Identification of the fragmentation mechanisms in wet-phase fine grinding in a stirred bead mill. *Chemical Engineering Science*. **52**: 3605-3612.

Verma, S., Kumar, S., Gokhale, R. and Burgess, D.J. (2010). Physical stability of nanosuspensions: Investigation of the role of stabilisers on Ostwald ripening. *International Journal of Pharmaceutics*. **406**: 145-152.

Yamada, T., Saito, N., Imai, T. and Otagiri, M. (1999) Effect of grinding with hydroxypropyl cellulose on the dissolution and particle size of a water-soluble drug. *Chemical and Pharmaceutical Bulletin*. **47**: 1311-1313.

Yokoyama, T., Tamura, H., Tsui, H. and Jimbo, G. (1996). Simulation of ball behaviour in a vibration mill in relation with its grinding rate: Effects of fractional ball filling and liquid viscosity. *International Journal of Mineral Processing*. **44-45**: 413-424.

York, P., Ticehurst, M.D., Osborn, J.C., Robert, R.J. and Rowe, R.C. (1998). Characterisation of the surface energies of milled dl-propranolol hydrochloride using inverse gas chromatography and molecular modelling. *International Journal of Pharmaceutics*. **174**:179-186.

Ziller, K.H. and Ruprecht, H. (1988) Control of crystal growth in drug suspensions. *Drug Development and Industrial Pharmacy*. **14**: 2341-2370.

Zillter, K.H. and Rupprecht, H.H. (1991). Control of crystal growth in drug suspensions. *Pharmazeutische Industrie*. **52**: 1017-1022.

Figures

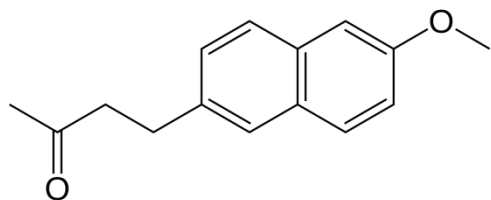


Figure 1. Structure of nabumetone

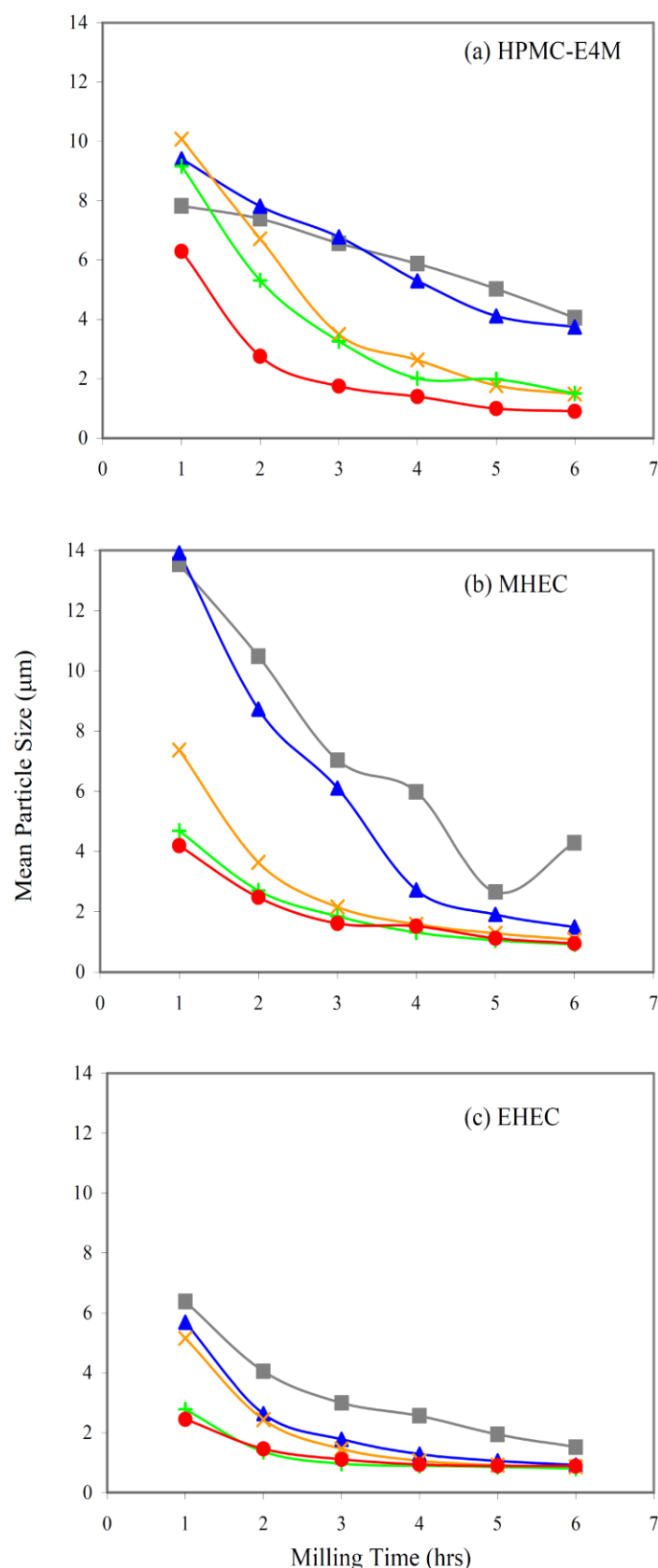


Figure 2. The variation in particle size determined using laser diffraction with milling time of 20 wt% nabumetone suspensions in the presence of various molecular weight of 1.5 wt% (a) HPMC(-EM4) (b) MHEC, (c) EHEC polymer solutions. CE degraded for 1 h (\blacksquare), 4 h (\blacktriangle), 8 h (\times), 16 h ($+$), 24 h (\bullet), $n = 1$. Viscosity average molecular weights (M_v) of the CEs are given in Table 2. No error bars are shown due to the results being obtained from one experiment, although it should be noted that the data are the mean of triplicate measurements of particle size using laser diffraction, a repeatability of around 3-5 % was routinely obtained in the measurements.

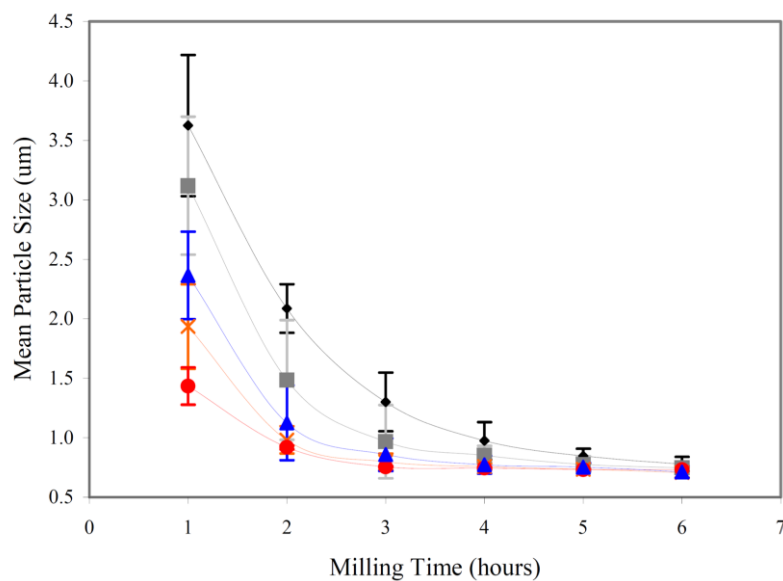


Figure 3. The variation in particle size determined using laser diffraction with milling time of 20 wt% nabumetone suspensions in the presence of various molecular weight of 1.5 wt% HPC(-EF) solutions undegraded M_v 112 Kg/mol (◆); HPC(-EF) degraded for 1 h, M_v 95 Kg/mol (■); HPC(-EF) degraded for 4 h M_v 80 Kg/mol (▲); HPC(-EF) degraded for 8 h, M_v 65 Kg/mol (×); HPC(-EF) degraded for 24 h, M_v 47 Kg/mol (●) ($n = 3 \pm sd$).

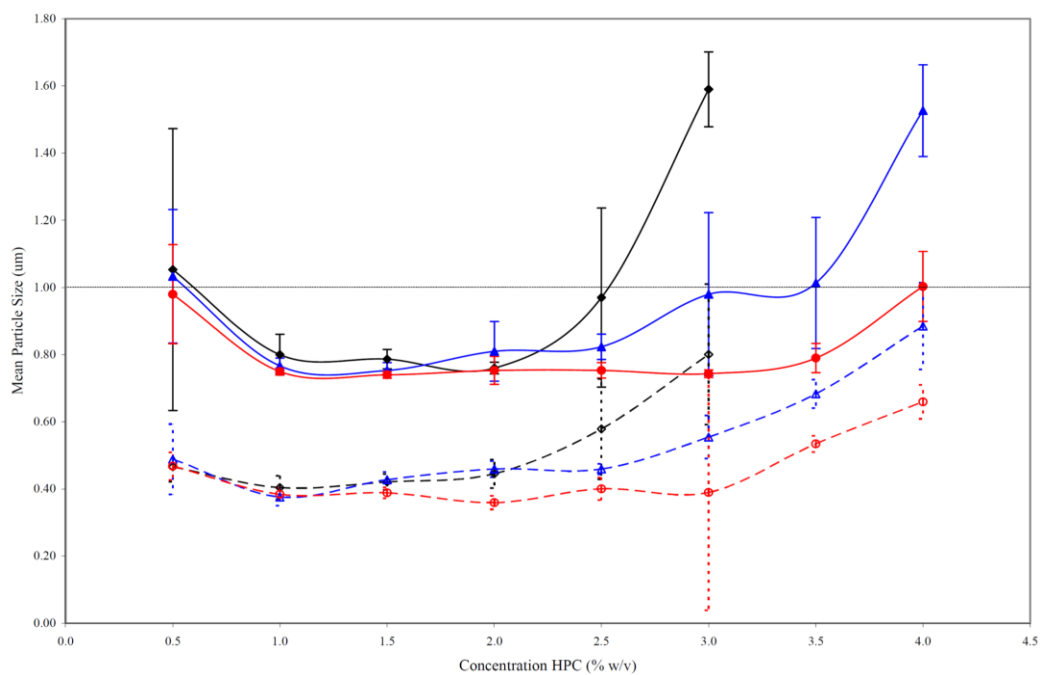


Figure 4. The effect of polymer molecular weight and concentration on the mean particle size obtained from laser diffraction (solid line) and photon correlation spectroscopy (dashed line) of nabumetone particles wet bead milled for six hours. HPC(-EF) undegraded, M_v 106 Kg/mol (\blacklozenge); HPC(-EF) degraded 4 h, M_v 89 Kg/mol (\blacktriangle); HPC(-EF) degraded 24 h, M_v 57 Kg/mol (\bullet) ($n = 3 \pm sd$).

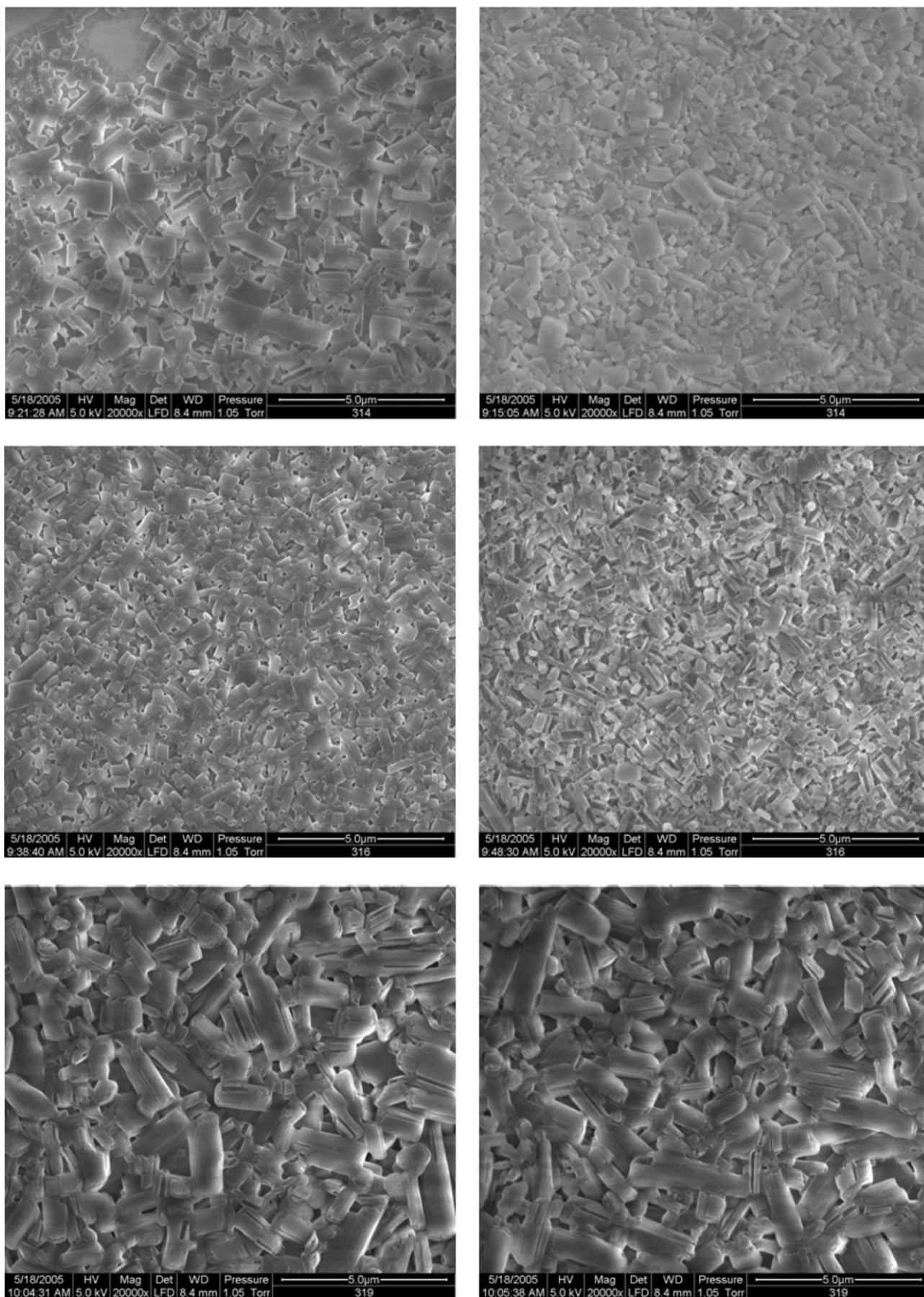


Figure 5a. Scanning electron micrographs of a nabumetone suspension wet bead milled for 6 h in the presence of (top) 0.5 wt%, (middle) 1.5 wt%, (bottom) 3.0 wt% HPC(-EF) undegraded, M_v 106 Kg/mol (x 20,000).

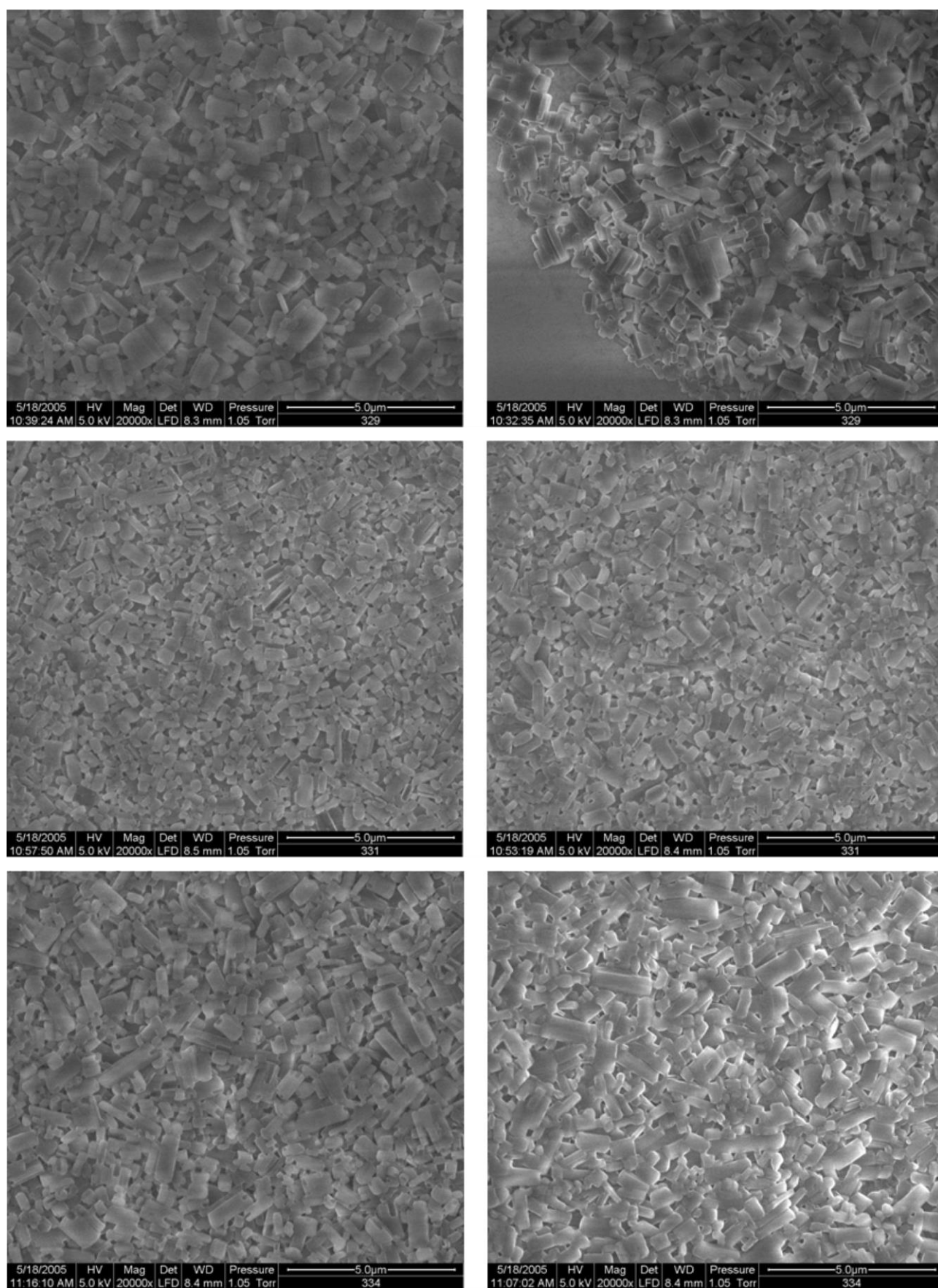


Figure 5b. Scanning electron micrographs of a nabumetone suspension wet bead milled for 6 h in the presence of (top) 0.5 wt%, (middle) 1.5 wt%, (bottom) 3.0 wt% HPC(-EF) degraded 4 h, M_v 89 Kg/mol ($\times 20,000$).

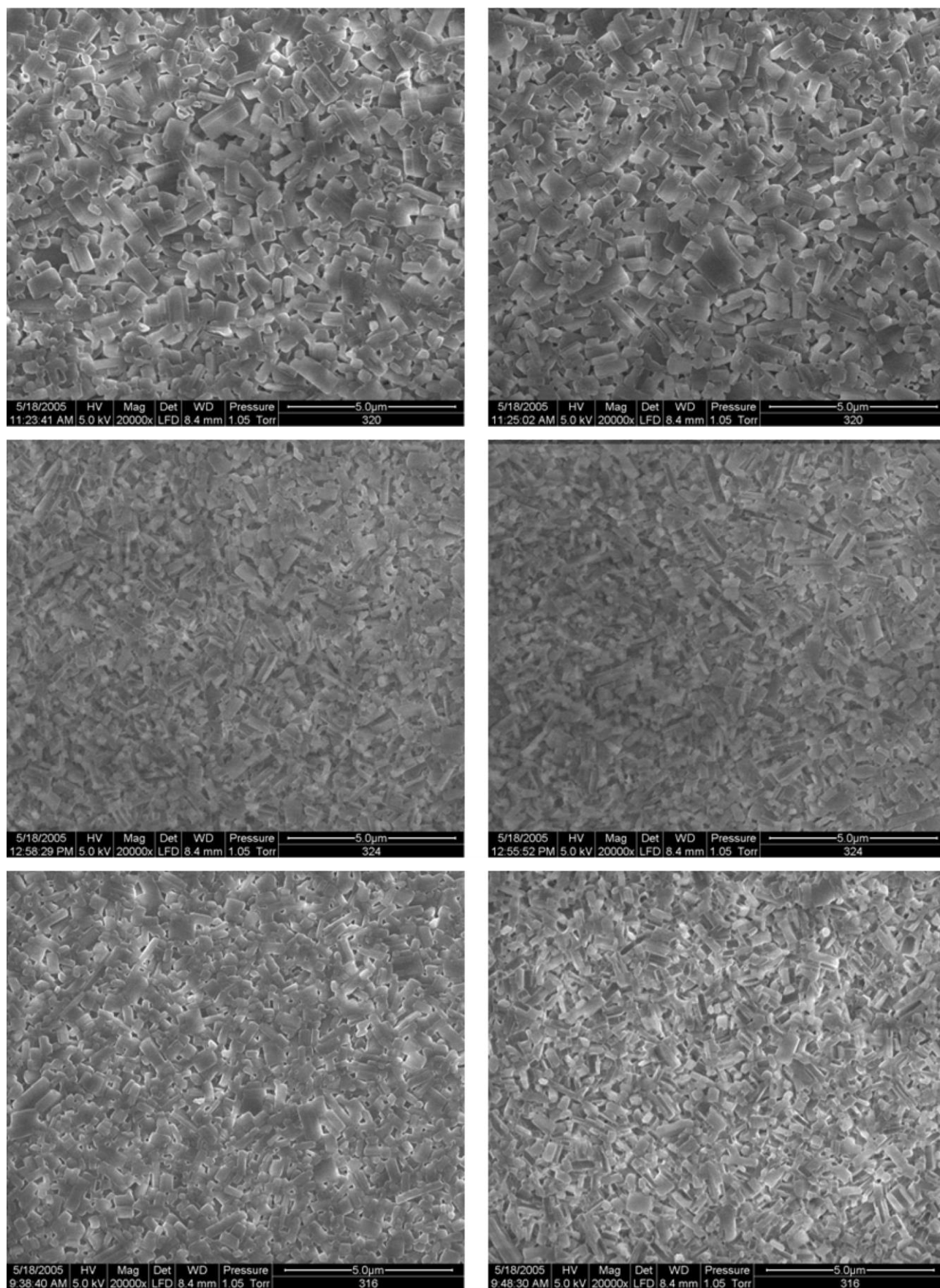


Figure 5c. Scanning electron micrographs of a nabumetone suspension wet bead milled for 6 h in the presence of (top) 0.5 wt%, (middle) 1.5 wt%, (bottom) 3.0 wt% HPC(-EF) degraded 24 h (M_v 57 Kg/mol) (x 20,000).

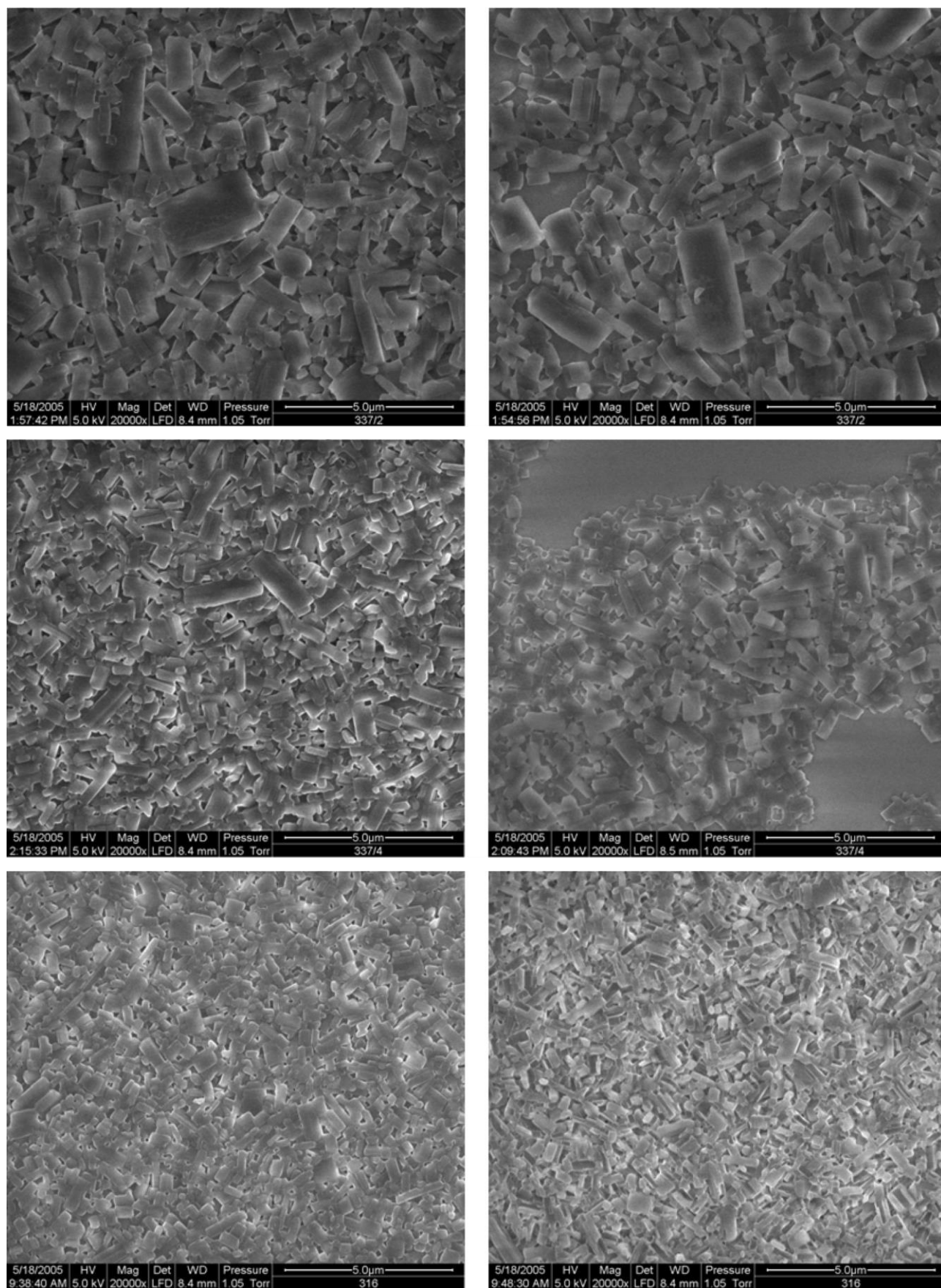


Figure 6a. Scanning electron micrographs of a nabumetone suspension wet bead milled for in the presence of 1.5 wt% HPC(-EF) undegraded (M_w 106 Kg/mol) for (top) 2 h, (middle) 4 h and (bottom) 6 h (x 20,000).

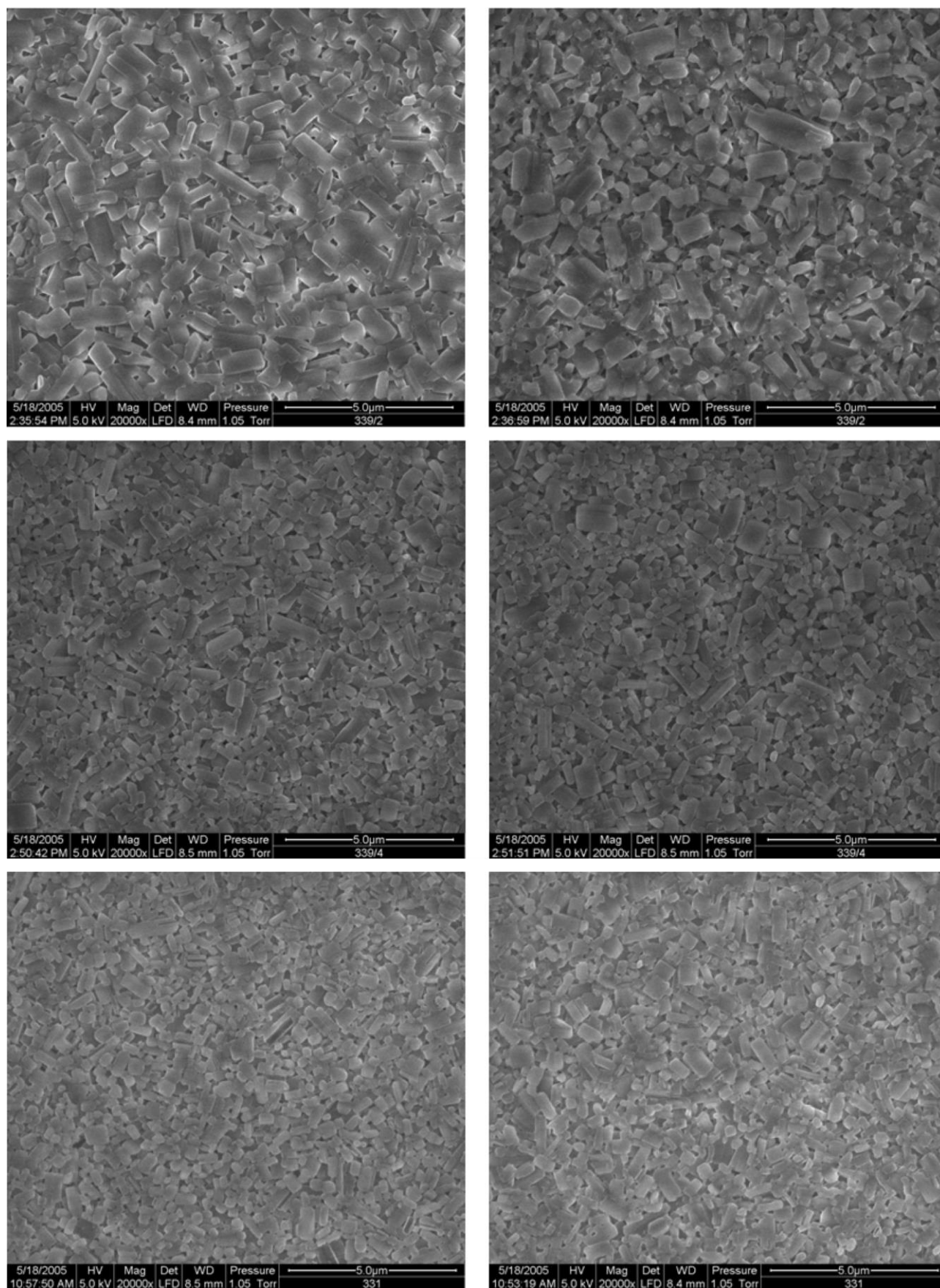


Figure 6b. Scanning electron micrographs of a nabumetone suspension wet bead milled for in the presence of 1.5 wt% HPC(-EF) degraded 4 h (M_w 89 Kg/mol) for (top) 2 h, (middle) 4 h and (bottom) 6 h (x 20,000).

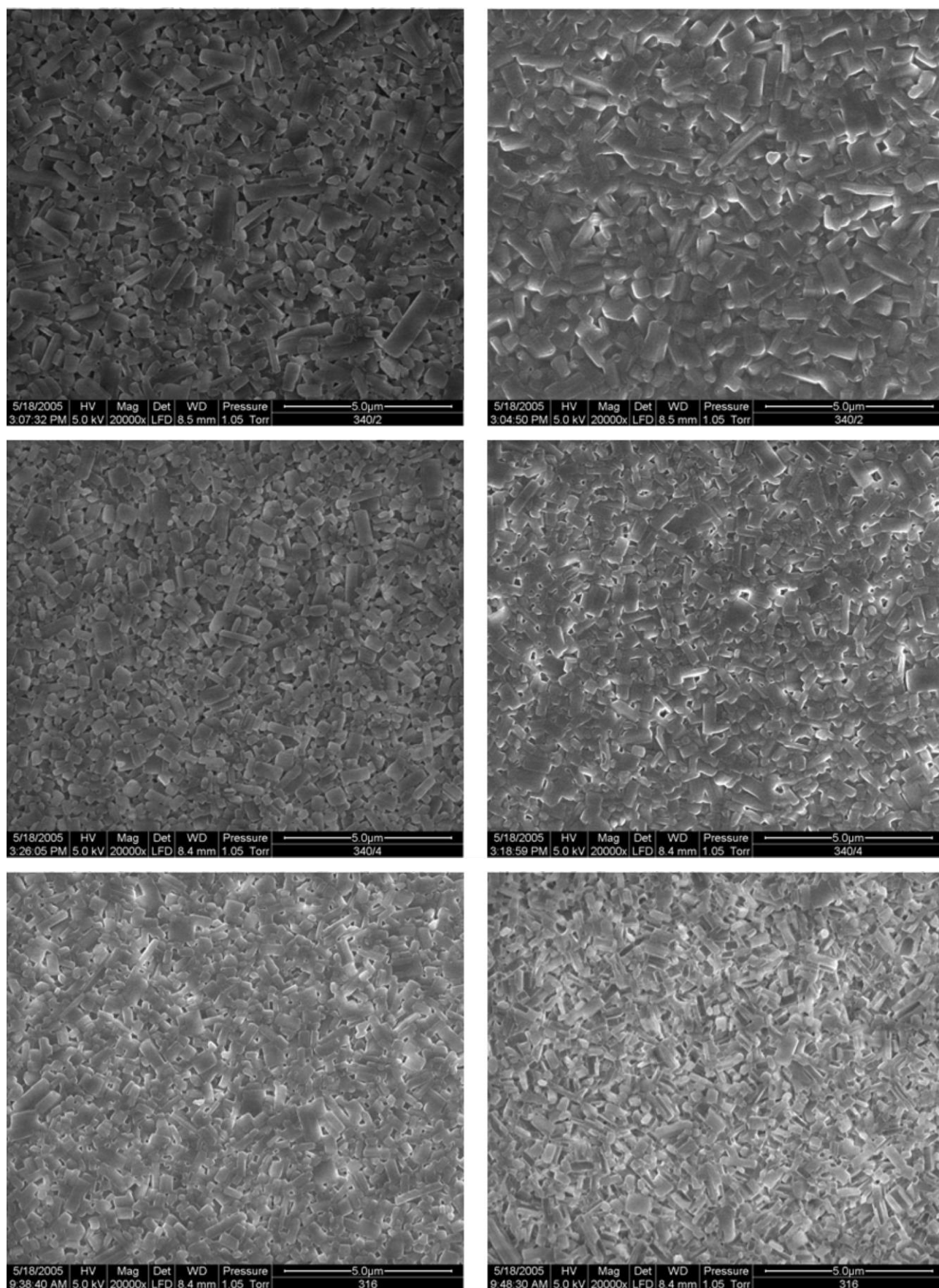


Figure 6c. Scanning electron micrographs of a nabumetone suspension wet bead milled for in the presence of 1.5 wt% HPC(-EF) degraded 24 h (M_v 57 Kg/mol) for (top) 2 h, (middle) 4 h and (bottom) 6 h (x 20,000).

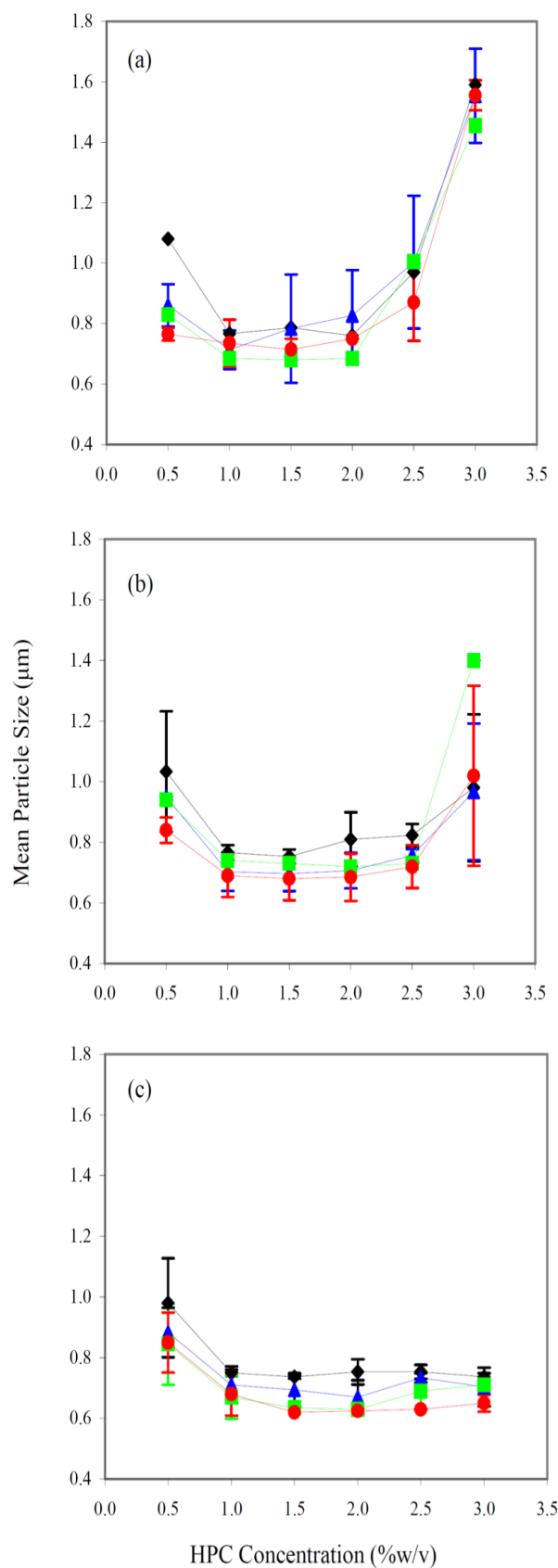


Figure 7. The variation in particle size obtained from laser diffraction of nabumetone suspensions milled in the presence of varying HPC(-EF) concentrations at a) M_n 106 Kg/mol, b) M_n 89 Kg/mol c) M_n 57 Kg/mol with storage time. Immediately after production (\blacklozenge); 6 months (\blacktriangle); 9 months* (\blacksquare); 12 months* (\bullet). ($n = 3 \pm \text{sd}$, * $n = 2$).

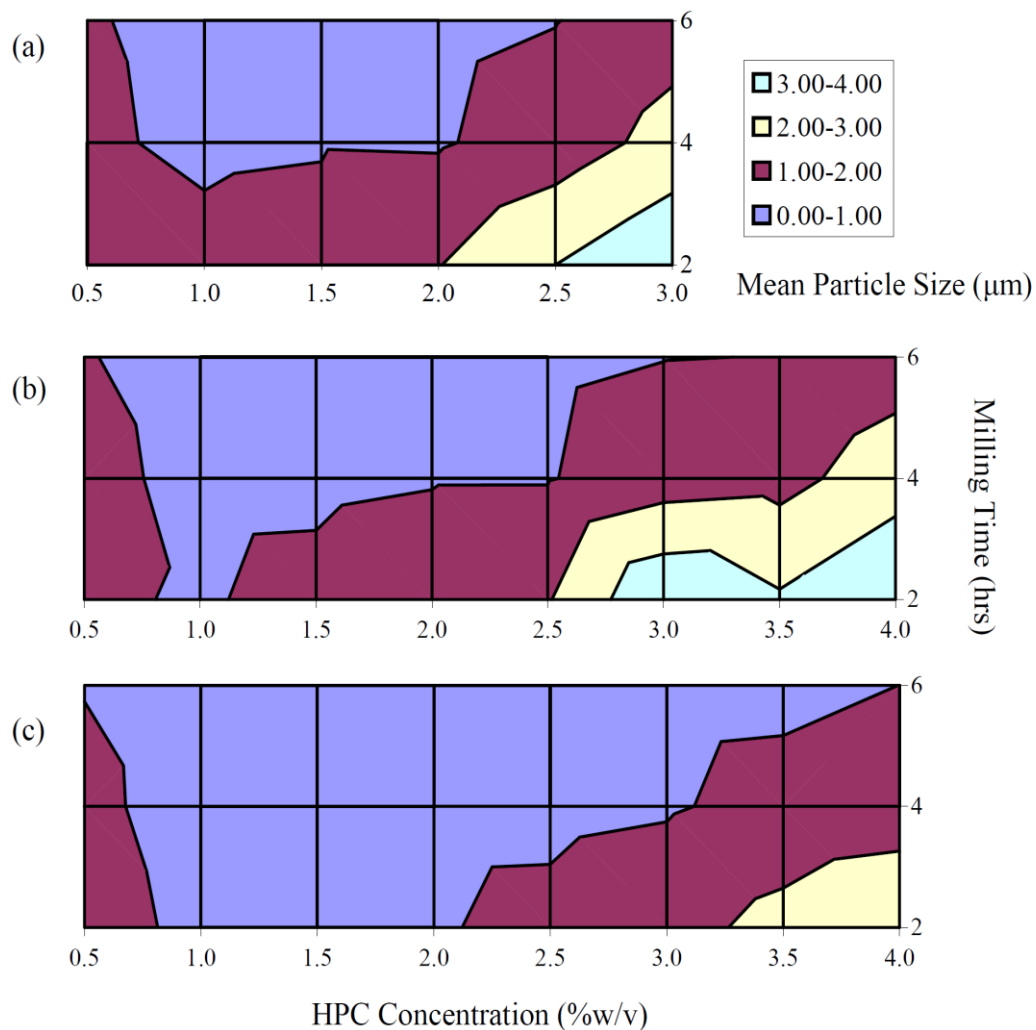


Figure 8. Contour plot of mean particle size obtained from laser diffraction against duration of wet bead milling and HPC(-EF) concentration at (a) undegraded, M_w 106 Kg/mol, (b) degraded 4 h, M_w 89 Kg/mol and (c) degraded 24 h, M_w 57 Kg/mol ($n = 3$).

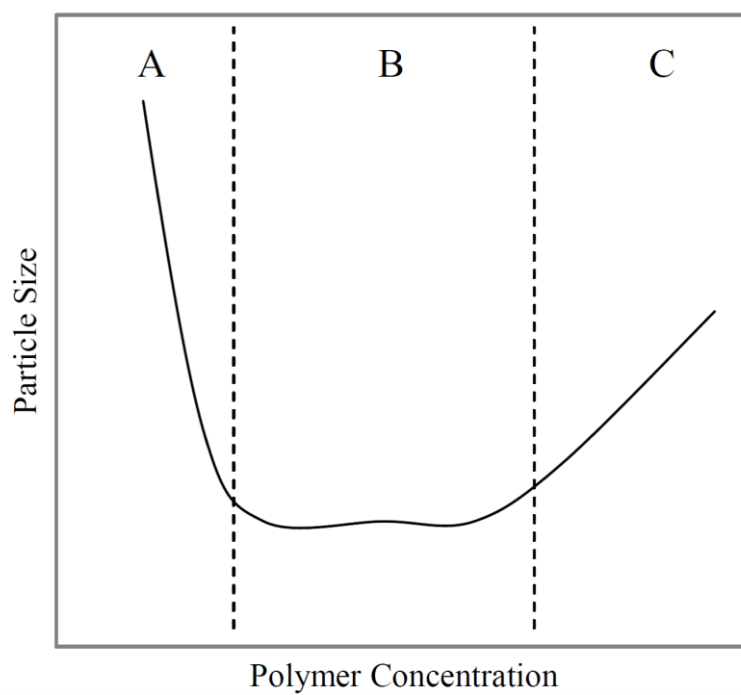


Figure 9. Generalisation of the effect of increasing polymer concentration on the particle size of wet bead milled drug particles.

Table 1. Structure of nonionic cellulose ethers studied

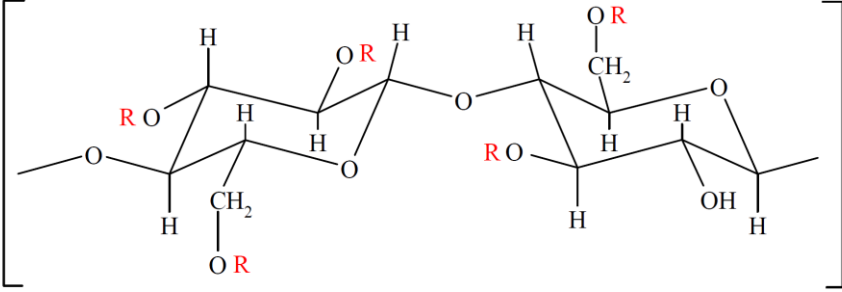
Cellulose Ether	Abbrvn.	Etherification Agent	Substituent R
			
Hydroxyethyl-	HEC	Ethylene oxide	$-(\text{CH}_2-\text{CH}_2\text{O}-)_n\text{H}$
Hydroxypropyl-	HPC	Propylene oxide	$-(\text{CH}_2\text{CH}(\text{CH}_3)\text{O}-)_n\text{H}$
Methylhydroxyethyl-	MHEC	Methyl chloride, ethylene oxide	$-\text{CH}_3, -\text{CH}_2\text{CH}_2\text{OH}$
Hydroxypropylmethyl-	HPMC	Methyl chloride, propylene oxide	$-\text{CH}_2\text{CH}(\text{CH}_3)\text{OH}, -\text{CH}_3$
Ethylhydroxyethyl-	EHEC	Ethyl chloride, ethyl chloride	$-\text{CH}_2\text{CH}_3, \text{CH}_2\text{CH}_2\text{OH}$

Table 2. Measured viscosity and viscosity (M_v) molecular weight of un-degraded and ultrasonically degraded cellulose ethers (Goodwin et al., 2011)

Cellulose Ether	HEC		HPC		MHEC		EHEC		HMPC	
	$[\eta]$ dL/g	M_v Kg/mol	$[\eta]$ dL/g	M_v Kg/mol	$[\eta]$ dL/g	M_v Kg/mol	$[\eta]$ dL/g	M_v Kg/mol	$[\eta]$ dL/g	M_v Kg/mol
Undegraded	4.48	236.0	1.09	112.2	2.56	N/D	4.19	114.0	7.7 0	382.7
Degraded 1 h	2.89	142.2	0.95	94.9	2.01	N/D	2.19	52.0	4.2 5	186.5
Degraded 4 h	1.75	79.9	0.82	79.4	1.68	N/D	1.29	26.8	3.3 4	139.4
Degraded 8 h	1.38	60.8	0.69	64.5	1.45	N/D	1.09	21.8	2.3 8	92.5
Degraded 16 h	0.96	40.1	0.60	55.2	1.20	N/D	0.88	16.6	1.6 6	59.8
Degraded 24 h	0.83	33.0	0.53	47.0	1.07	N/D	0.79	14.5	1.6 6	38.8

*Mark-Houwink parameter not available for MHEC, hence only $[\eta]$ quoted.

Table 3. The effect of wet bead milling time on the number averaged (M_n) and weight averaged molecular weight (M_w) of unadsorbed HPC with nabumetone (n=1).

Sample	Duration of Milling (h)	M_n (kg/mol)	M_w (kg/mol)	P_d
HPC-EF UnD	0	40.3	110.0	2.8
	2	43.4	114.5	2.7
	4	36.5	92.7	2.6
	6	32.1	66.5	2.1
HPC-EF USD4h	0	ND	79.4*	ND
	2	34.9	82.0	2.4
	4	31.7	72.0	2.3
	6	29.8	55.0	1.9
HPC-EF USD24h	0	29.5	55.4	1.9
	2	27.7	51.1	1.9
	4	25.9	45.4	1.8
	6	25.7	44.2	1.7

* M_v

ND = not determined

†The results for un-degraded samples refer to separate samples and were not from the same stock solution as those milled.