ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2014; 5(3): 53 - 57

ORIGINAL RESEARCH ARTICLE

In-Situ Fiber Optics Analysis of MicroparticlesofaPoorly Water Soluble Drug

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Received 04 Feb 2014; Revised 25 May 2014; Accepted 09 Jun 2014

ABSTRACT

Erroneous results during drug release study can seriously affect the study outcomes. The principle aim of this study was to understand the use of in-situ fiber optic UV analysis for in-vitro release testing of ibuprofen (model drug) microparticle (IbM) in reducing these erroneous results. IbMwere prepared by melt fusion technique. Characterization of the prepared IbM involved particle size analysis, scanning electron microscopy (SEM) and micromeritic properties. In-vitro drug release was analysed both by finer optics system and conventional method of drug analysis. All results were statistically analysed at $p< 0.05$. Results showed the micron range for the prepared microparticle. SEMrevealed the structure of the drug and polymer mixture. Percentage drug release obtained using fiber optics analysis was statistically significant ($p=0.0051 \pm 0.0021$), and higher as compared to that obtained using the traditional method. This difference was attributed to loss of microparticle during the pipetting (separation process), and manual errors in the traditional analysis method. Fiber optics dip probe technique could be a new and better way for analysis of micro-formulations.

Keywords: Microparticle; fiber-optics; in-situ; optical microscopy; traditional method.

INTRODUCTION

Particledispersions or solids sized 1-1000 µm are microparticles.[1]Microparticle manufacturing as vaccine and anticancer drug carriers is history. They now enhance the efficiency of the drug-delivery, boost release profile, and assist in drug targeting.[2]Different routes of administration, modification of particle contours, and site-specific targeting enables microparticles to be an efficient drug-delivery system. Microparticle formation permits alteration and enhancement of a molecule's characteristics.[3]

UV fiber-optics has drawn significant interest within the pharmaceutical industry for in-situ dissolution analysis. The fiber-optics varies in the types of spectrometers, and the probes.[4]Latest publications and research work support the notion that UV fiber optics is a breakthrough for dissolution testing.[5]Intensive manual labour or online dissolution systems coupled with spectrophotometric analysis are the mainstay of in-vitro dissolution, and leads to unwanted errors at any stage. Fiber-optics alters this conventional approach. The fiber-optics delivers the UV spectrometer to the sample solutions, which is better than delivering the samples to a UV spectrometer. An in-situ fiber optics analysis a real-time drug release level without the need for sample removal.Its convenience, higherdata-collection speed, high information density, and relative ease of using enables the fiber-optics method to become valuable than the standard techniques of dissolution testing.[6] Fiber-optics has attained importance from a novel technology to a validated procedure in quality control areas.[7]

Ibuprofen(Ib) ((RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid) is a well-known non-steroidal anti-inflammatory drug exhibiting antipyretic and analgesic activity.[8]Apart from general side-effects, the seriously affected portionis the gastro intestinal tract. Bleeding and ulceration are common. These side-effects are due to the solubility issues, besides the large dose ingested for the drug.[9]Consequently, there is a need to minimize such negative effects, and extend the drug's anti-inflammatory activity.

Therefore, the present study involved the fiber optic dissolution analysis of ibuprofen

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microparticles (IbM). The results were compared to the traditional method of dissolution testing.

MATERIALS:

Ib was a gift sample from National Pharmaceutical Industry, Rusayl, Musact, Oman. Steric acid, ethanol, sodium dihydrogen phosphate, disodium hydrogen phosphate, hydroxy propyl methyl cellulose (E15 LV) was procured from Chemistry for Life, Muscat. Double distilled water was used throughout the experiment.

EXPERIMENTAL

Preparation of ibuprofen stearic acid microparticles

Ib and stearic acid were weighed in different ratiosviz 1:2, 1:3 and 1:4. They were melted in a pre-heated (85^{o}C) reaction vessel. De-ionized water (1L) was heated to 85^{o}C on a hot - plate and added to the molten mix. This was then left for 5 minutes at 85^{o}C and stirring then commenced. Experiments were conducted with continous stirring. After 5 minutes, the heating element of the water bath was switched off, allowing the temperature of the system to fall. Cold water (5^{o}C) was circulated around the system, cooling down the resulting emulsion in less than 15 minutes. When the temperature of the system reached 30^{o}C, the stirrer was switched off and the resulting microparticle collected by suction filtration using a Büchner filter. The solid product collected was dried overnight in a vacuum desiccator over silica gel. The preparations were coded as follows 1:2 (F1), 1:3 (F2), and 1:4 (F3).

Physicochemical Characterizations

Particle Size Analysis

The particle size of the prepared formulation is essential to understand the formulation properties. The particle size of the prepared IbM were assessed in optical microscope (Zeiss Primo Star, Jena, Germany).

Percentage Yield

The dry microparticles were weighed and the percentage yield of the different ratios of microparticles were calculated by the formula

$$Percentage\ Yeild = \frac{Weight\ of\ microparticles}{Weight\ of\ Polymer+Drug} \times 100 \text{----}(1)$$

Drug Loading and Encapsulation Efficiency

Drug loading and encapsulation efficiency was determined for thedifferent ratios of microparticles using the following formulas:

$$Drug\ loading = \frac{Weight\ of\ drug\ in\ microparticles}{Weight\ of\ microparticles} \times 100\text{----}(2)$$

$$Entrapment\ efficiency = \frac{Actual\ weight\ of\ drug\ in\ sample}{Theoretical\ weight\ of\ drug} \times 100\text{-} \quad (3)$$

Micromeritics

Particle size of pure drug was determined by microscopic method using calibrated ocular micrometer. The bulk and tapped density of the different ratios of microparticles were measured using densitometer.Further Carr's index, Hausner's ratio and angle of repose of pure drug and different ratios were determined.

Scanning Electron Microscopy (SEM)

The SEM analysis provides valuable and highly detailed information about the sample including external morphology (texture), chemical composition, crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample. Pure Ib and different ratios of IbM were subjected to SEM analysis. Sampleswere mounted in 10mmx10mm aluminum stub by sticking the sample on double sided carbon adhesive disc. The mounted sampleswere transferred to Auto Fine Coater (JFC-1600) to sputter coat the sample with platinum. The platinum coated samples were screened under SEM (JSM-6510LA, Tokyo, Japan).

In Situ Fiber Optic UV Monitoring

Transmission Dip Probe (T300-RT, Ocean Optics, Florida, USA) paired to UV spectrometers (Ocean Optics QE65 Pro, Florida, USA) was utilized for absorbance measurement. The light supply was deuterium from a UV-VIS-NIR lightsource (Ocean Optics DH-2000-BAL, Florida, USA). These probes are helpful for in-situ, real time sample tracking. Two solarization resistant 300-µm optical fibers are a part of the T300-RT-UV-VIS Transmission Dip Probe. One fiber is for illumination and the other for reading. Fiber optics is effective as it needs zero warm time, and low maintenance and electrical energy. Photodegradation is prevented, as samples are not exposed to UV light. The light beam is intensive, and therefore, the fiber optics system has great noise efficiency. It has a synchronised reference modification {and thus can keep peak integrity at each scan speed. A superb photometric linearity guarantees accurate data and its reproducibility, and removes the necessity for dilution prior to measurement. For the experiment, an integration time (shutter speed) was set at 100 milliseconds, boxcar width set to 10, scans to average readings to 10. The data was accumulated through Ocean Optics software (Spectrasuite®, Ocean Optics Inc., Florida, USA). The integration time was kept

higher (upto 85% of the detector capacity) because the detector would monitor the incoming photons for longer time. The scans to average were kept at 10 to give bestsignal to noise ratio. The boxcar width was kept to 10 giving smoothness to the data and a good signal to noise ratio.

Drug Release Study

A reliable drug holding egg membrane (dialysis membrane) was removed from a fresh egg, and utilized during drug release study in phosphate buffer pH 7.4. Phosphate buffer pH 7.4 being a medium of drug's greatest solubility was favored as the dissolution media. The membrane was tied to two clamps, and the pure Ib, and the IbM were put separately. The release study was carried out for 360 minutes. One milliliter sample was taken at designated time intervals, suitably diluted using phosphate buffer, pH 7.4, and analyzed by fiber optics, and conventional UV spectrophotometer (Shimadzu 1800, Shimadzu, Tokyo, Japan) at 272 nm.

RESULTS AND DISCUSSION

The results of particle size are represented in (**Table 2**). The results showed statistical significance ($p = 1.232 \pm 0.435$) at a confidence level set at 5% ($\propto = 0.05$), which meant that any change in the drug mixtures property, would solely be due to the resulting combination and not due to the surface area. However, there was a slight size difference, which could be either due to excess drug or the polymer in the mixture.

The percentage yield of the prepared microparticles was calculated according to equation. The percentage yields of microparticles of different ratios are as shown in (**Table 1**)**.**A 100% yield cannot be achieved due to reasons of adhesion of materials on the stirrer, and loss during filtration. It was observed that as the drug to polymer ratio in the formulation increased, the yield increased. The drug content of F3 (90%) was highest and F1 (82%) was lowest.

The drug loading and encapsulation efficiency were calculated according to equation 2 and 3 respectively.The drug loading and encapsulation efficiency in the different ratios of microparticles are as shown in Table1.The drug loading of the different formulations were found to be between 16.25% - 24.2%. The drug loading was found to be highest for F2. The increase in drug loading can be due to increase in particle size resulting in high entrapment efficiency. This was further confirmed by particle size analysis. Furthermore, the encapsulation efficiency was highest for F2. Initially the encapsulation efficiency increased as the ratio of stearic acid increased. This can be due to the availability of sufficient amount of stearic acid to entrap the drug. However, the decrease in F3 could be attributed to the accumulation of excess of stearic acid preventing the drug from moving inside.

The micromeritic properties like bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose were determined as shown in (Table 2).

The prepared microparticles were in the range between 62.51-63.8µm. The higher ratio of stearic acid was associated with increased microparticle size, although not significant. This clarifies higher drug loading and higher encapsulation efficiencies of F2. Carr's index of pure drug was 30.3. The indexes of F1, F2 and F3 range from 7.14-11.11 indicating excellent flow. The Hausner's ratio for the formulation was found to be in the range 1.07-1.13, indicating excellent flow properties as compared to the value of pure drug (1.43). The values of different formulation fell within the acceptable limits of angle of repose. Among all the three formulations F2 was the best.

Scanning electron microscopy

Along with the other studies, scanning electron microscopy (SEM) was also used to observe the surface changes of the microparticles. The SEM image of different ratios of IbM is shown in (**Fig 1-3**). The particles were irregularly shaped. Upon close magnificationit was seen that the particles were not smooth which may be attributed to the encapsulating material.

***In-vitro* Drug Release Study**

The in-vitro drug release (Figure 4) analysed by fiber optics revealed that the F2 formulation was good not only sustain but also release the drug in a controlled manner. The F2 formulation had a significantly ($p=0.0051 \pm 0.0021$, $p<0.05$) different release profile as compared to the pure drug with a dissimilarity factor ($f_1=22$), and F3 ($p=0.0431 \pm 0.0011$, $p<0.05$) with a dissimilarity factor ($f_1=32$). Although the F1 was able to release 50.2% as compared to 54% of F2 (statistically insignificant, $p=1.2310 \pm 0.5432$, $p<0.05$) with a similarity factor of ($f_2 = 93$), which would be ideal for a 12 hourly release profile, however considering the drug loading and efficiency F2 was considered to be the best

formulation.The comparison of the traditional method with the fiber optics showed a significant difference ($p=0.01312 \pm 0.0132$, $p<0.05$) in the drug release profile. In general, they were lower readings, which resulted in errors. The reason could be attributed to the loss in the drug amount during sampling, dilution and subsequent analysis. We observed similar significant difference ($p=0.0365 \pm 0.0109$, $p<0.05$) in the reproducibility of the absorbance results.

Table 1: Data representing Percentage yield, Drug Loading and Encapsulation Efficiency

Formulation Code	Percentage Yield (%)	Drug Loading (%)	Encapsulation Efficiency (%)
F1	82.43 ± 1.23	23.9± 1.43	71.7± 2.54
F2	88.05± 1.65	24.2± 0.98	97.6±1.41
F3	90.65 ± 1.54	16.25±1.54	81.25±0.89

Table 2: Micromeritic properties of the prepared formulation

Formulation Code	Particle Size (*μm*)	Carr's index	Hausner's ratio	Angle of repose (°)
Pure Drug	63.45± **2.43**	30.3±**1.52**	1.43 ± **2.65**	41.18± **1.65**
F1	63.51± **1.76**	7.14±**0.98**	1.07±**2.01**	23.02± **1.98**
F2	63.8±**2.21**	7.65±**1.54**	1.08± **1.98**	27.2± **1.54**
F3	63.86±**1.87**	11.11±**2.0**	1.13 ±**2.87**	28.25± **1.21**

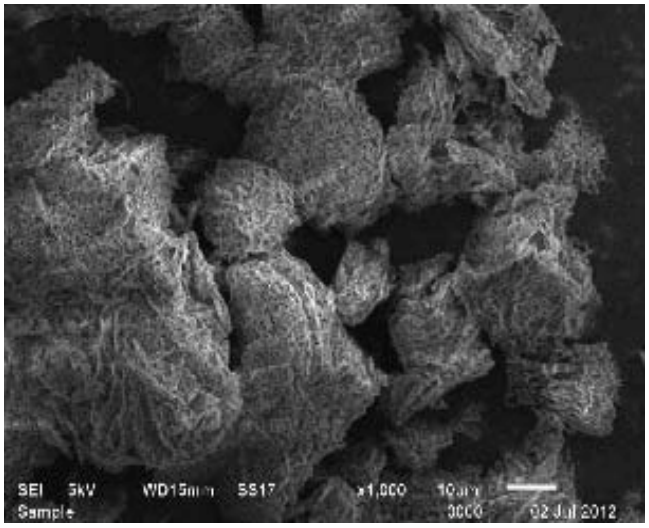

Figure 1: SEM image of ibuprofen stearic acid microparticles prepared in the ratio 1: 2

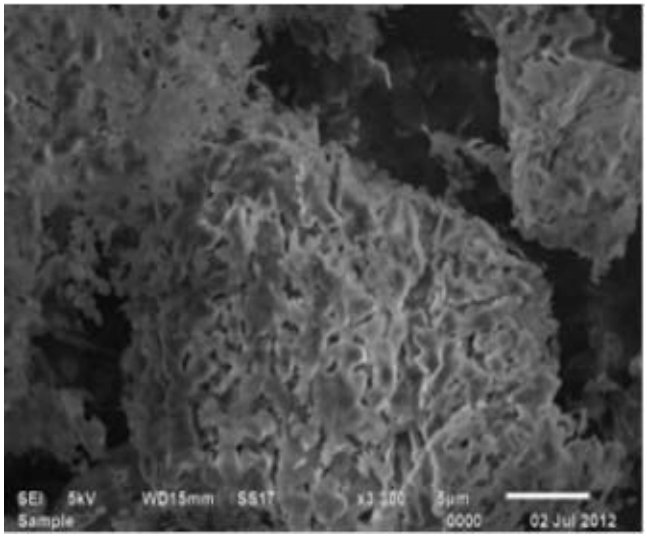

Figure 2: SEM image of ibuprofen stearic acid microparticles prepared in the ratio 1:3

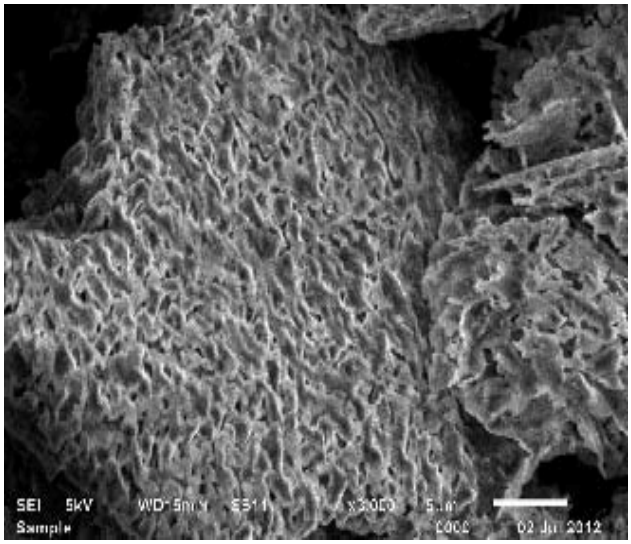

Figure 3: SEM image of ibuprofen stearic acid microparticles prepared in the ratio 1: 4

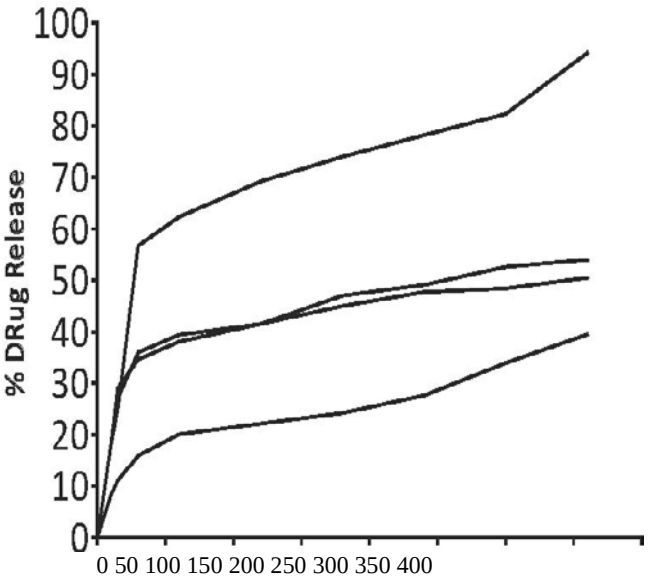

Figure 4: Drug release profiles of all the formulation along with the pure drug

CONCLUSION

IbM were successfully prepared, characterized, and evaluated using a fiber optics system. The fiber optics system produced reliable results, and was a great tool in terms of simplicity, without causing any significant difference as compared to traditional analysis results. The commonly used analysis method suffered from some flaws including significantreproducibility difference in inter-day as well as intra-day results. Fiber optics could be a very helpful tool in drug release study in the future.

ACKNOWLEDGEMENT

We would like to acknowledge the Research Council Grant (ORG/HSS/12/004), and Dr. Anil Philip for his interpretation of fiber optics results, and allowing to use his funded laboratory. We would also like to thank DARIS, University of

Nizwa, Oman for SEM results. We would like to thank Thurayia Al Hazri, Moza Al Mahrouqi, Juhaina Al Hinai for their contribution in the study.

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