



J. Chem. Pharm. Res., 2010, 2(3):433-452

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Evaluation of glutinous rice starch based matrix microbeads using scanning electron microscopy

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ABSTRACT

The microbeads were prepared by an industrially feasible micro orifice ionotropic-gelation method using glutinous rice starch from Assam Bora rice, and sodium alginate backbone with different crosslinking agents. The microbeads fabricated through several preformulation trials were evaluated in reference to drug release, drug excipient compatibility and mucoadhesive potential. The effect of various formulation and processing parameters was studied by scanning electron photomicrographs taken before and after dissolution. Photomicrographs provides the information about the surface texture, size, mechanistic properties, suitability of drying condition and mechanism of drug release from the prepared micro devices.

Key words: Microbeads, scanning electron microscopy, drug delivery, glutinous starch.

INTRODUCTION

The biological diversity responds to a number of new, emerging concerns including thrust for the research and developments especially in the field of biotechnology, life sciences, chemical sciences, traditional phytopharmaceuticals and natural products [1]. The major focus of the research carried out on the value of genetic resources has been on their use in the pharmaceutical and agricultural industries, which use genetic diversity as a source of information in their development of new products. Economists have long analyzed the research and development

process as one of information utilization, application and diffusion. The concept of research and development is usually presented as a production process itself dependent upon a stock of "information" for its generation of useful innovations. The study was undertaken to explore the utility of *Assam Bora rice* in pharmaceutical drug carrier systems, as the researchers from Assam Agriculture University reported that the bora rice is a variety of glutinous rice having composed of mainly amylopectin and only a traces of amylase [2].

Excipients are primarily used as diluents, binders, disintegrants, adhesives, glidants and sweeteners in conventional dosage forms like tablets and capsules. The traditional view that excipients are inert and do not exert any therapeutic or biological action or modify the biological action of the drug substance has changed and it is now recognized that excipients can potentially influence the rate and/or extent of absorption of a drug [3-5]. In this connection the bora rice polysaccharide is studied for its mucoadhesive potential and property of modulation drug release from the pharmaceutical drug carrier systems. The several trial formulations of matrix microbeads were prepared and optimized through drug release and allied pharmacotechnical characterization. The aim of this study was to observe and evaluate the micro-morphology of prepared microparticulate systems using scanning electron microscopy (SEM) as the electron microscopy projections has become a well-established technique to obtain structural information about size and surface properties, and correlating the processing factors over the last 20 years [6-7]. The present paper uses scanning electron microscopy to study the surface characteristics, size, size distribution and other pharmacotechnical parameters and to study the effect of various process and formulation variables on fabricated drug carrier system.

EXPERIMENTAL SECTION

2.1 Materials: The bora rice was procured from the local village near about Dibrugarh University and was confirmed by local people. Drug samples were obtained as gift sample from industrial sources. All other chemicals used were the analytical grade laboratory reagents and were used as such without further testing.

2.2. Instruments and equipments: The following instruments were used: Balance: PB303 Delta Range (METLER TOLEDO, Switzerland), Digital mechanical stirrer (Remi Motors, Mumbai), Magnetic stirrer with hot plate (Macro scientific works®, Delhi), Hot air oven (NSW, India), Six Stage Dissolution Apparatus (Campbell Electronics, Mumbai), Optical Microscope (Olympus, Japan), UV-VIS Spectrophotometer: U-2001 (Hitachi Inc), Infrared Spectrophotometer (Perkin Elmer-883 IR spectrophotometer), Scanning Electron Microscope (HITACHI, S – 3600 N, Japan), Differential Scanning Calorimeter (METLER TOLEDO, Switzerland), X-Ray Diffraction (X-pert, Philips Powder XRD, Generator PW-117, XRD Control PW-118, U.K.), Digital P^H Meter (Model No-MP-220, METLER TOLEDO, Switzerland), FTIR (Perkin Elmer-883 FTIR spectrophotometer), Phase Contrast Microscope (Labomed XLR C II, India), Grinder-Mixer (Philips-HL 1618, India. Ltd), HPLC (SHIMADJU, Japan), Twin arm Shaker (Remi Motors, Mumbai.), Vacuum oven (Tempo Instruments and Equipments. Bombay), Ultrasonicator (Roop Telesonic ultrasonix Ltd), MAC Deionizer (MSW 198, CA-G 05), Homogenizer (Remi Motors, Mumbai), Cyclomixer (Remi Motors, Mumbai.)

2.3. Preparation of Microbeads: The drug loaded microbeads were prepared by the micro orifice ionotropic-gelation technique in all aqueous system. The carrier backbone was prepared by the pregelatinized bora rice along with sodium alginate in varying ratio [4,8]. Many preformulation trials were undertaken to optimize the preparation process for higher drug load and to accommodate the maximum percentage of bora rice producing beads with sufficient mechanical strength and acceptable pharmacotechnical parameters.

2.4. Size and size distribution: The particle size and size distribution of the beads was studied through optical and scanning electron microscopy. The particle size measurements were also carried out by the phase contrast microscope (LaboMed XLR II).

Table 1

Sample Codes for Scanning electron microscopy	
Blank beads cross-linked with Ca ⁺⁺ (2:1 <i>Bora rice</i> : sodium alginate ratio) oven dried:	FB
Drug Loaded beads cross-linked with Ca ⁺⁺ (1:1 Polymer: Drug) & (1:1 <i>Bora rice</i> : sodium alginate ratio) oven dried:	A
Drug Loaded (1:1 Polymer: Drug) & (2:1, <i>Bora rice</i> : sodium alginate ratio) with Ca ⁺⁺ Cross-linking: Oven dried Open air dried Vacuum dried	B C D
Drug Loaded beads cross-linked with Ca ⁺⁺ (1:1 Polymer: Drug) & (2:1 <i>Bora rice</i> : sodium alginate ratio) oven dried: AFTER DISSOLUTION: In 0.1 M HCl In Water In buffer pH 7.2	E F G
Drug Loaded (1:1 Polymer: Drug) & (2:1, <i>Bora rice</i> : sodium alginate ratio) with Ba ⁺⁺ Cross-linking: Oven dried	H
Drug Loaded beads cross-linked with Ba ⁺⁺ (1:1 Polymer: Drug) & (2:1 <i>Bora rice</i> : sodium alginate ratio) oven dried: AFTER DISSOLUTION: In 0.1 M HCl In Water In buffer pH 7.2	I J K
Drug Loaded beads cross-linked with AL ⁺⁺⁺ (1:1 Polymer: Drug) & (2:1 <i>Bora rice</i> : sodium alginate ratio) oven dried:	L
Drug Loaded beads cross-linked with Ca ⁺⁺ (1:1 Polymer: Drug) & (2:1 <i>Bora rice</i> : sodium alginate ratio) oven dried, Coated with HPMC:	M
Drug Loaded beads cross-linked with Ca ⁺⁺ (1:1 Polymer: Drug) & (2:1 <i>Bora rice</i> : sodium alginate ratio) oven dried, Coated with HPMC: In 0.1 M HCl In Water In buffer pH 7.2	N O P

2.5. Drug loading: The amount drug entrapped in the fabricated microbeads was estimated by extracting the drug from beads and its subsequent spectrophotometric quantification. Drug entrapment efficiency was calculated using the following formula.

$$\text{Entrapment efficiency} = \frac{\text{Estimated percentage drug loading}}{\text{Theoretical percentage drug loading}} \quad (100)$$

2.6. Mucoadhesion testing: The test for mucoadhesion was carried out using *in vitro* wash-off test reported by Lehr *et al.* 1992 [9].

2.7. Drug Excipient compatibility: The Drug excipient compatibility of the beads was analyzed through FTIR, DSC, XRD and Ultraviolet spectroscopy.

2.8. *In vitro* dissolution testing: The *in vitro* drug release study was conducted by USP XXIV dissolution test apparatus Campbell electronics, Mumbai) using basket type apparatus. The sample aliquots were withdrawn at predetermined intervals and were quantified spectrophotometrically to know the amount of drug released in relation to time. The study was conducted in triplicates and dissolution profiles were compared for reproducibility before they were used for modeling and characterization [8].

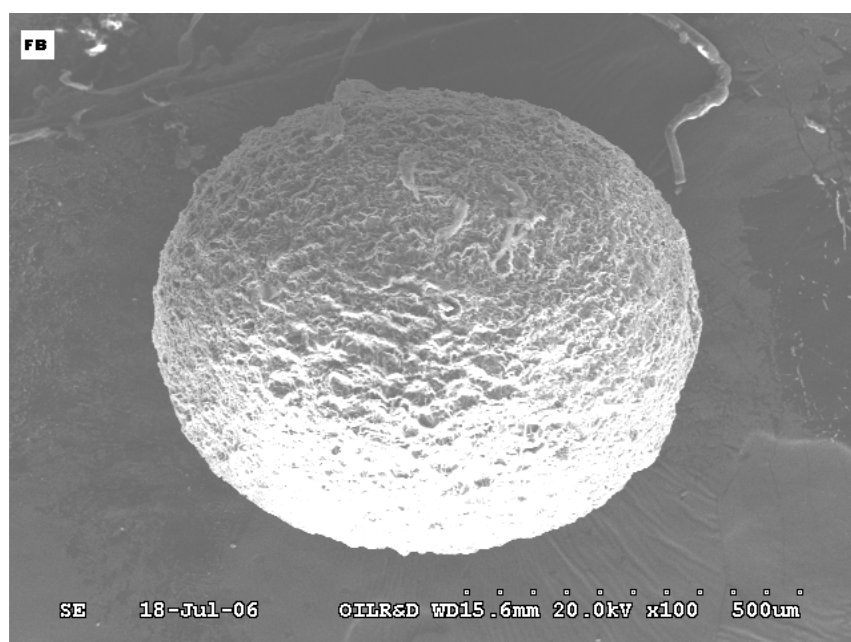
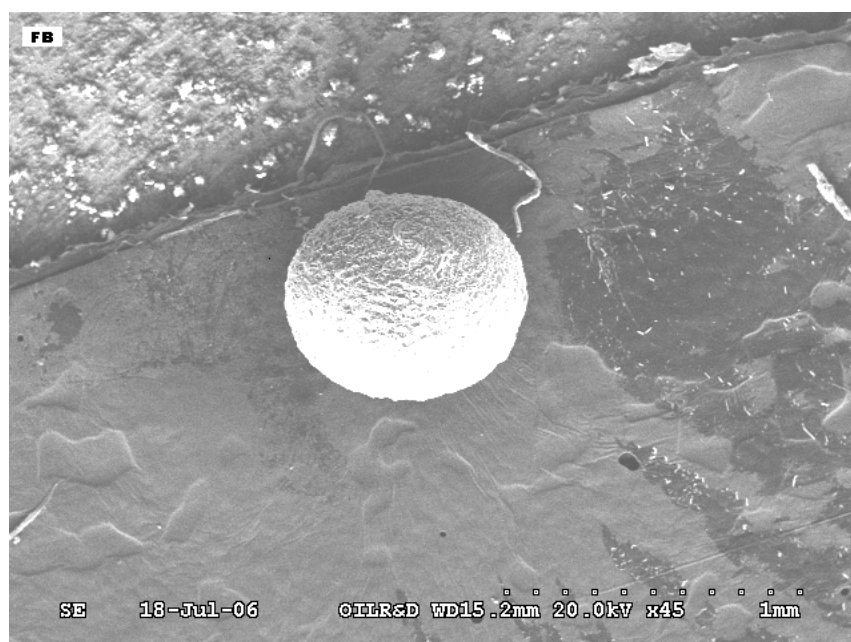
2.9. Scanning electron microscopy: The surface morphologies of the blank microspheres, drug loaded microspheres and microspheres collected after dissolution study, were investigated by using HITACHI, S – 3600 N, Scanning Electron Microscope at 15kv. Prior to examination samples were gold coated under vacuum (Fine coat, ion sputter, JFC-1100) to render them electrically conductive.

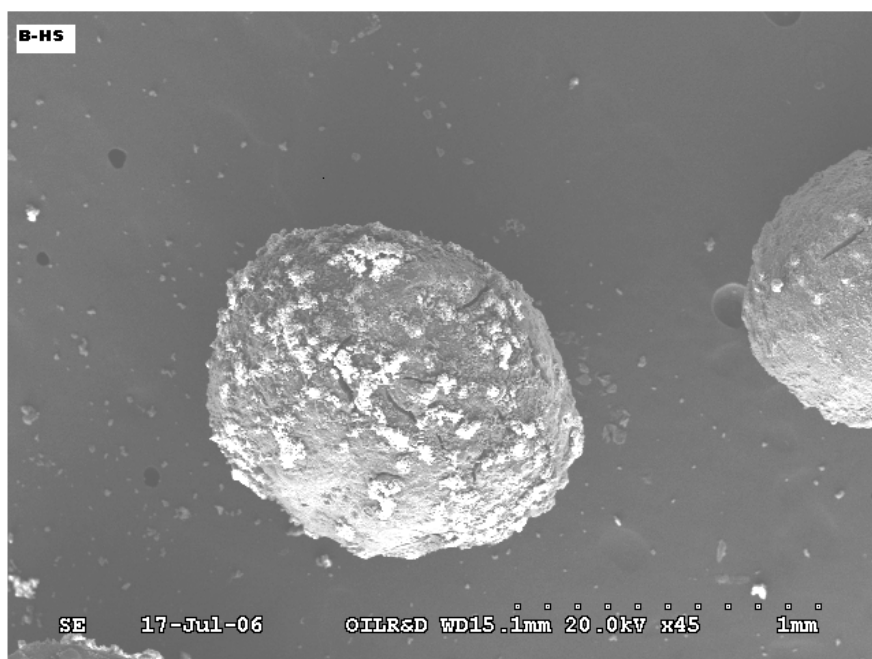
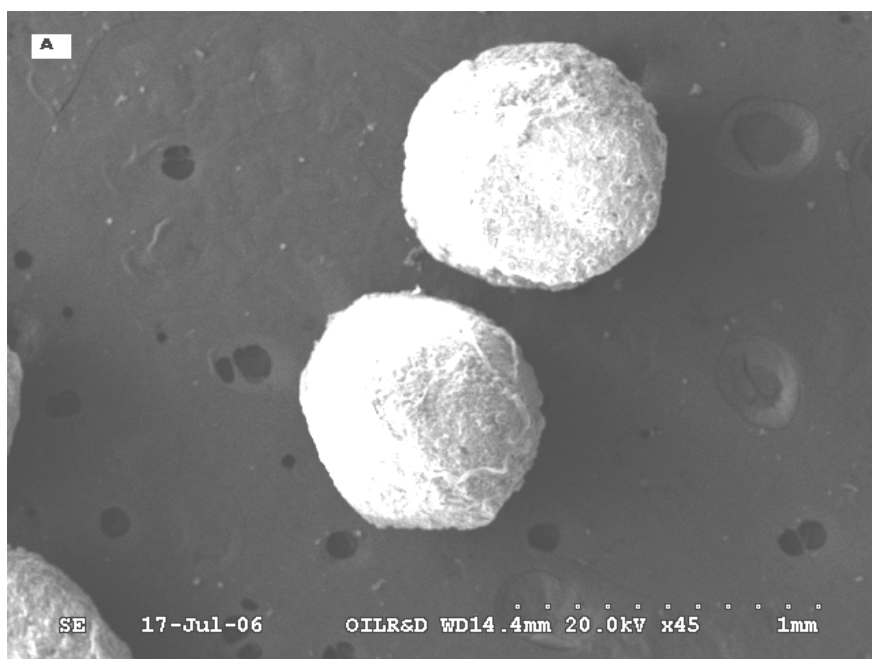
RESULTS AND DISCUSSION

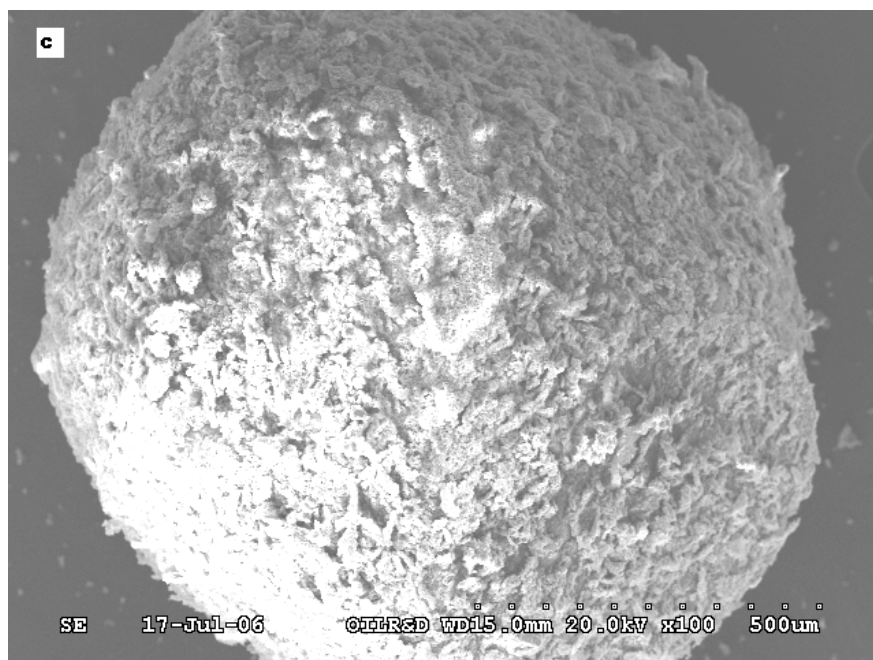
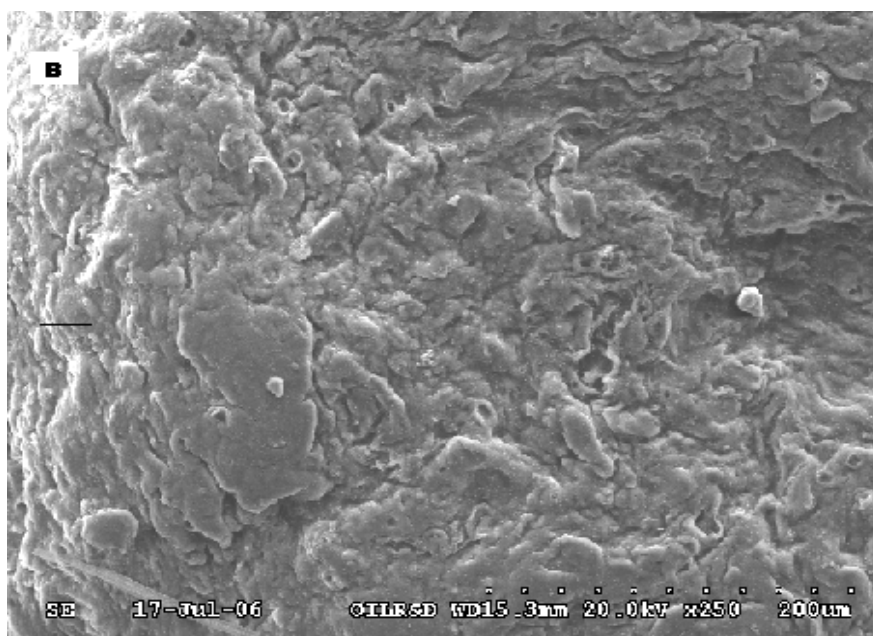
3.1. Pharmacotechnical characterization: The drug loaded microcarrier systems prepared and optimized through several preformulation trials using ionotropic gelation technique. The microbeads were almost spherical, discrete and free flowing in a size range of about 0.726 ± 0.008 mm to 1.16 ± 0.009 mm as shown in phase contrast and scanning electron microscopy. The percentage drug loading of beads was found to be in range of 19 – 53 % that is affected by some process parameters like drug polymer ratio, stirring speed, curing time, gel strength and medium of microencapsulation. The bora rice beads showed slow wash-off than that of the microspheres prepared by non-bioadhesive material. It is observed that the pH of the medium is important for hydration, solubility and adhesion of polymer to mucus membrane. Drug and excipient compatibility study have given no clue about any chemical interaction between the medicament and polymer backbone. The trial formulations were sorted on the basis of drug release behaviour and the potential formulations having acceptable release profile were taken forward for further characterization [10].

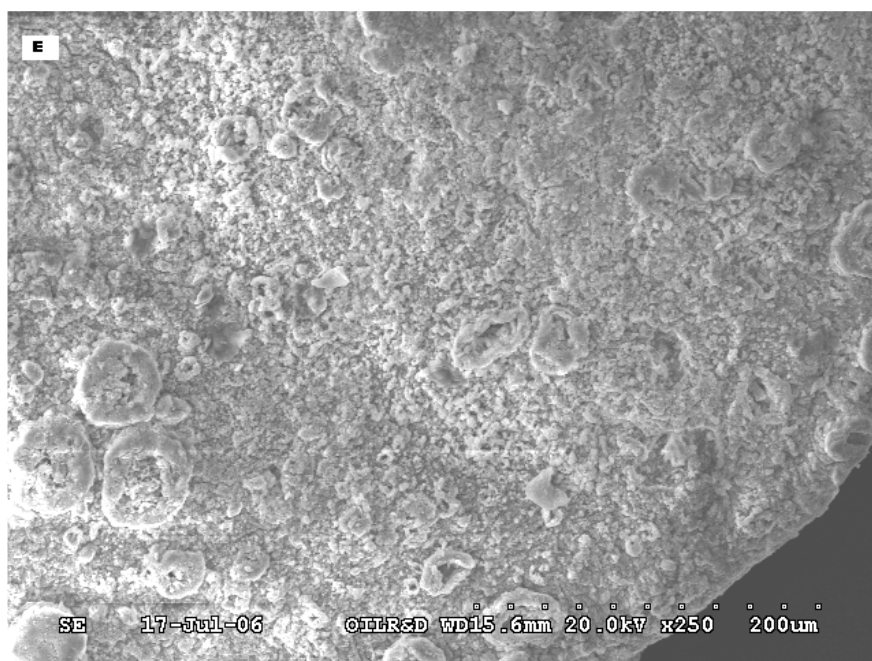
3.2. Scanning electron microscopy: The SEM photographs of the gel beads prepared with different formulation composition and processing variables (Table 1) are shown in the Fig 1. The scanning electron microscopy revealed that the prepared microbeads were almost spherical in shape and had a rough surface. The effect of drying condition, cross-linking agent, and polymeric coating were observed on the morphological features of the drug loaded and exhausted beads using the scanning electron microscopy [6]. The ratio of two polymers over the morphological characteristics was also comparable (Fig. 1: A and B). The scanning electron microscopy was performed on to the formulations prepared with the 2: 1 polymer/drug ratio that

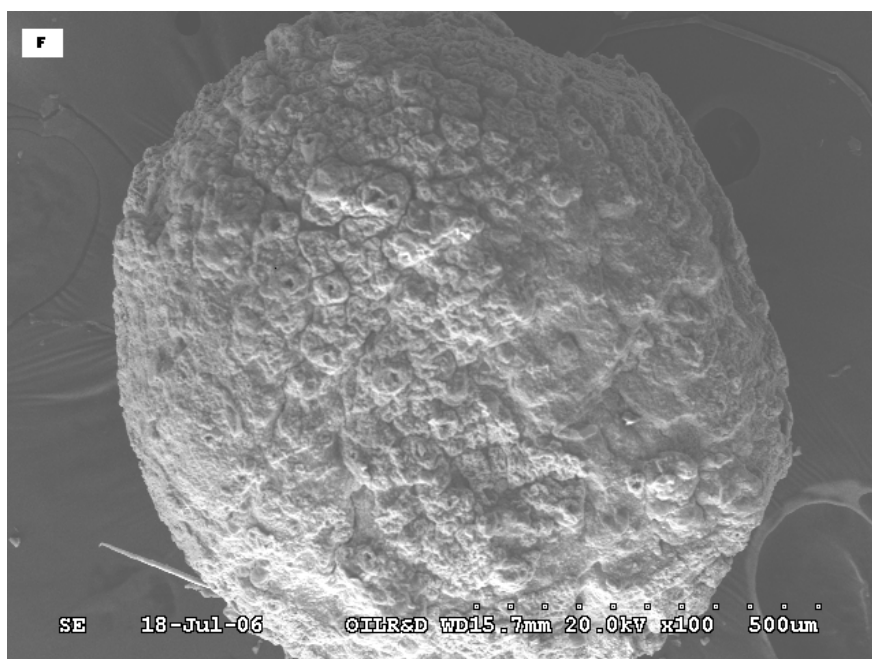
Fig. 1: Scanning Electron Micrographs of the beads

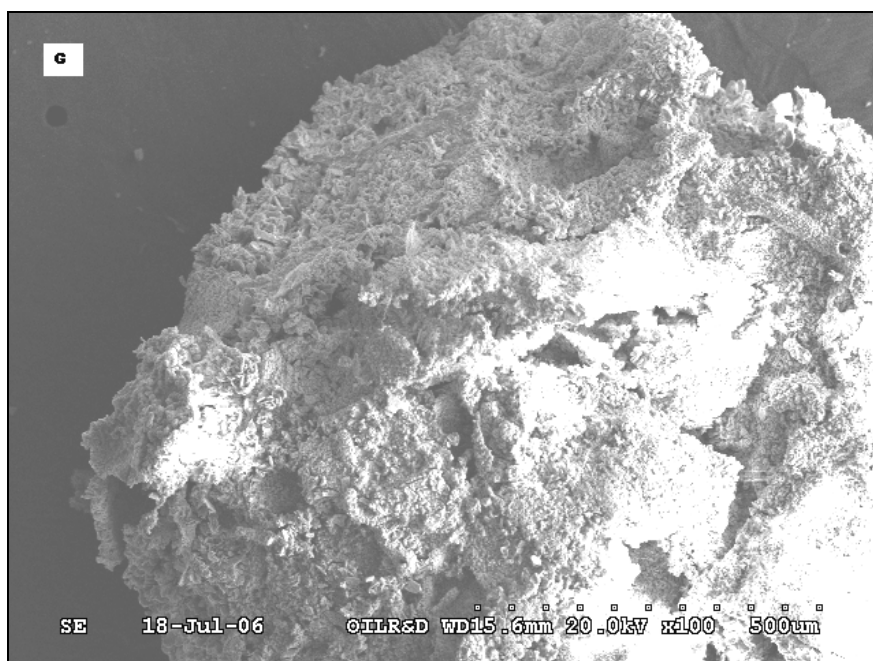


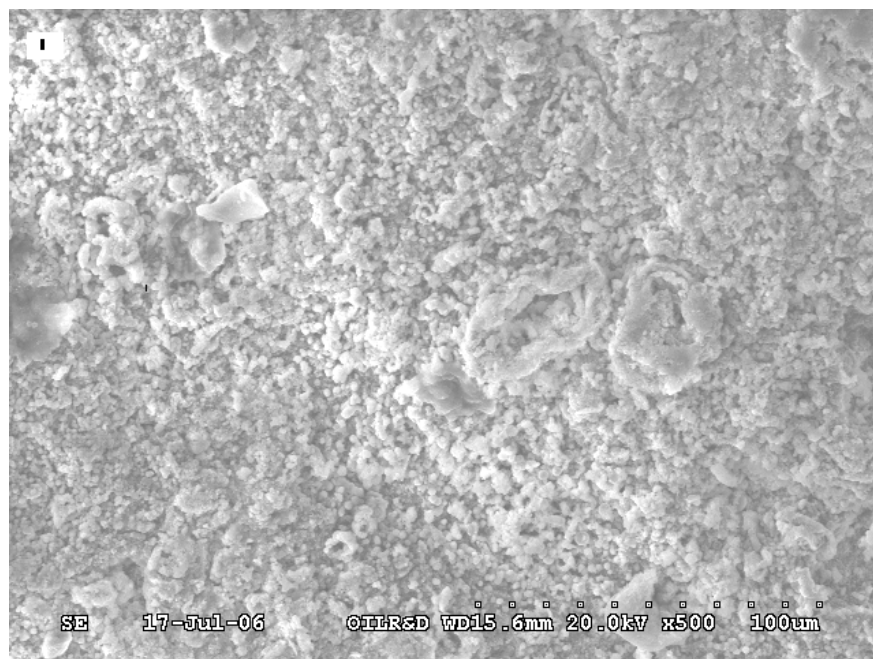


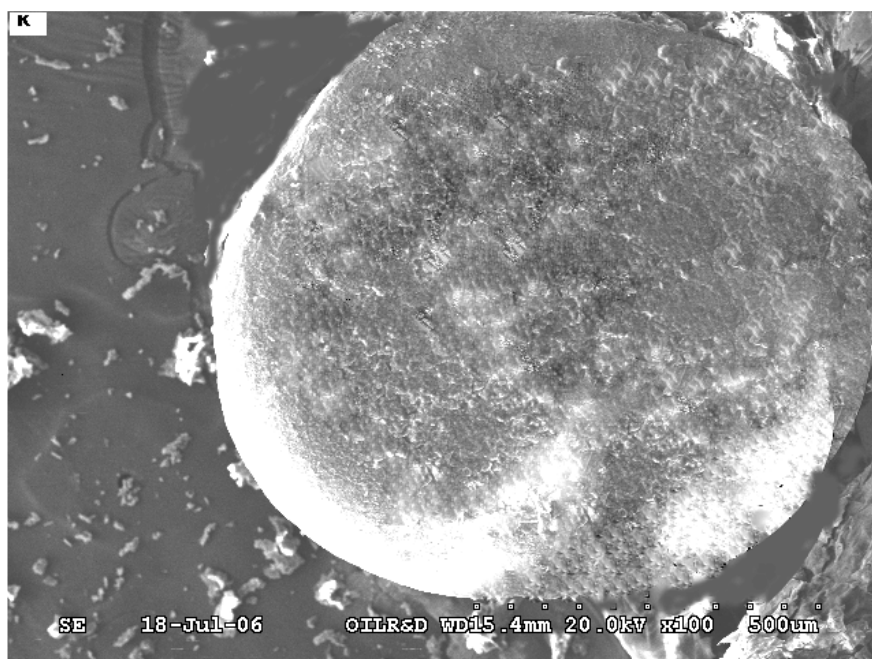
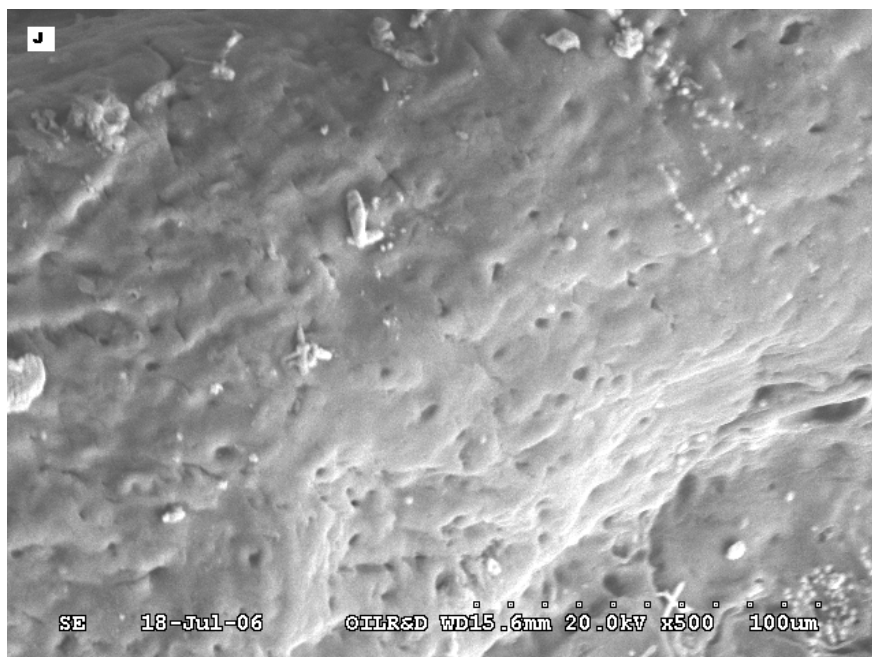


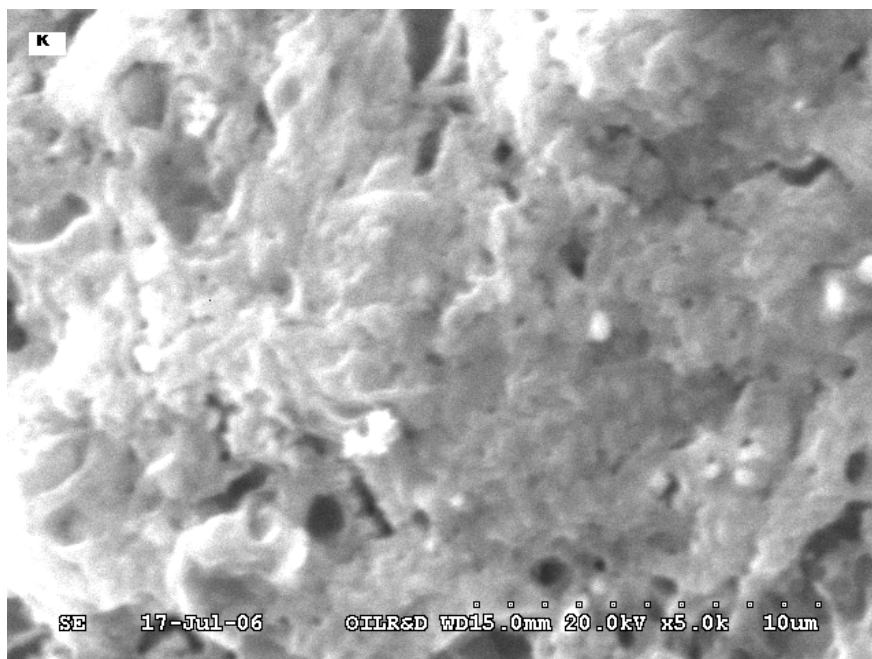


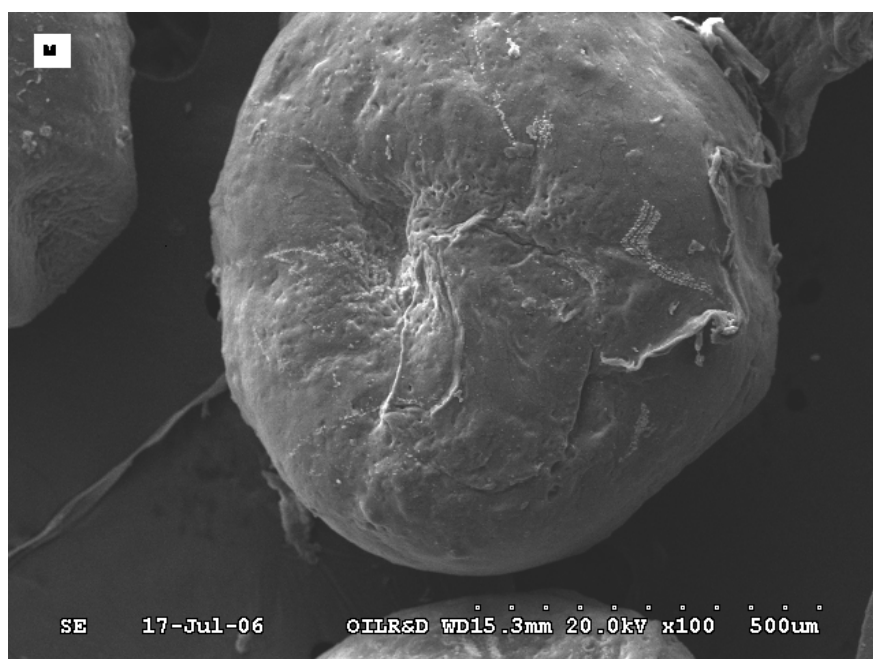
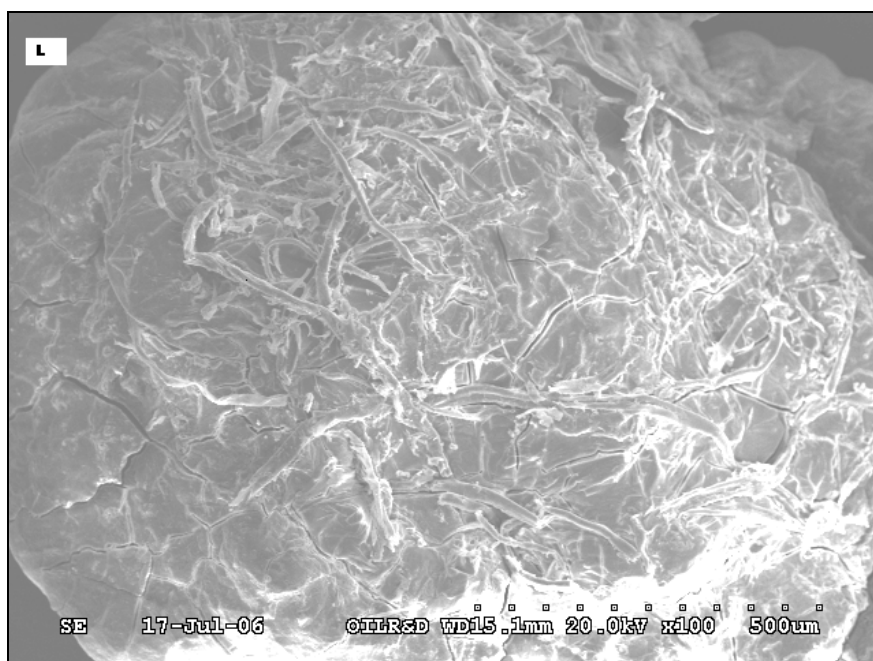


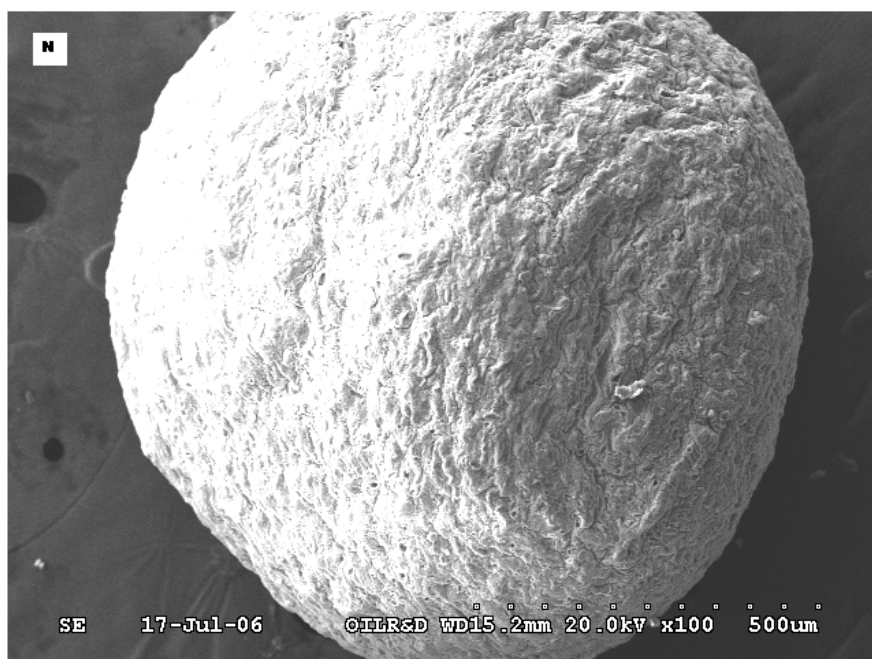
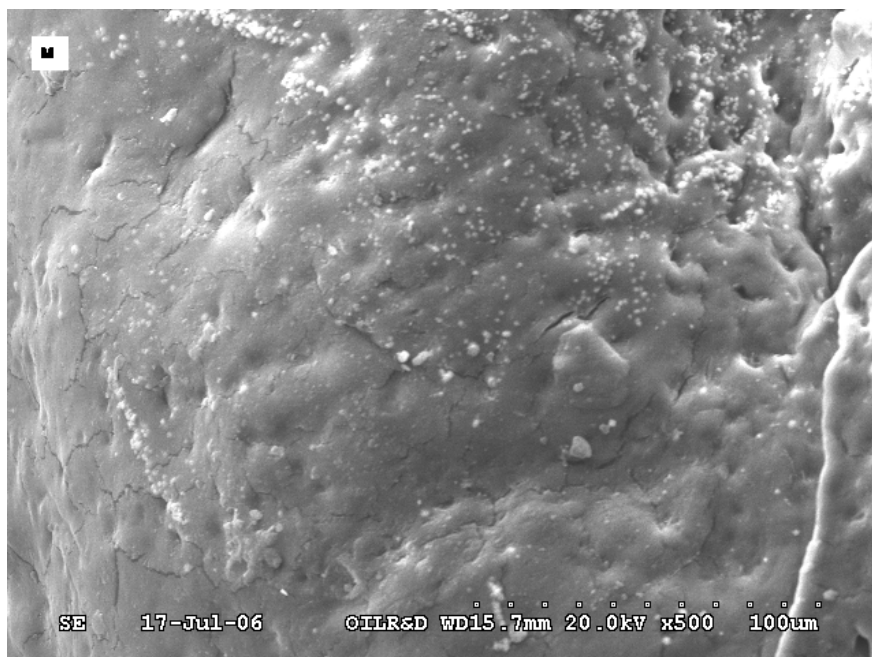


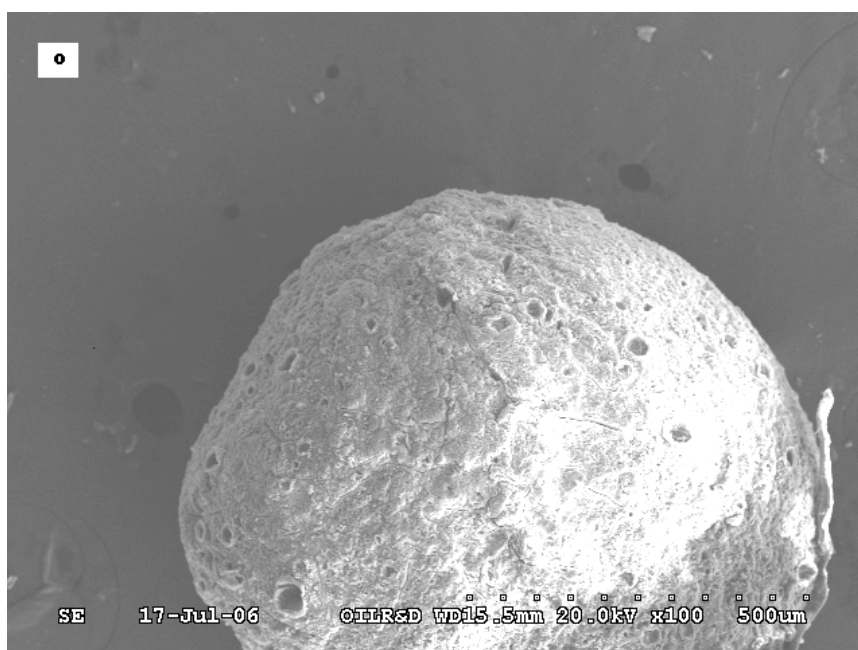
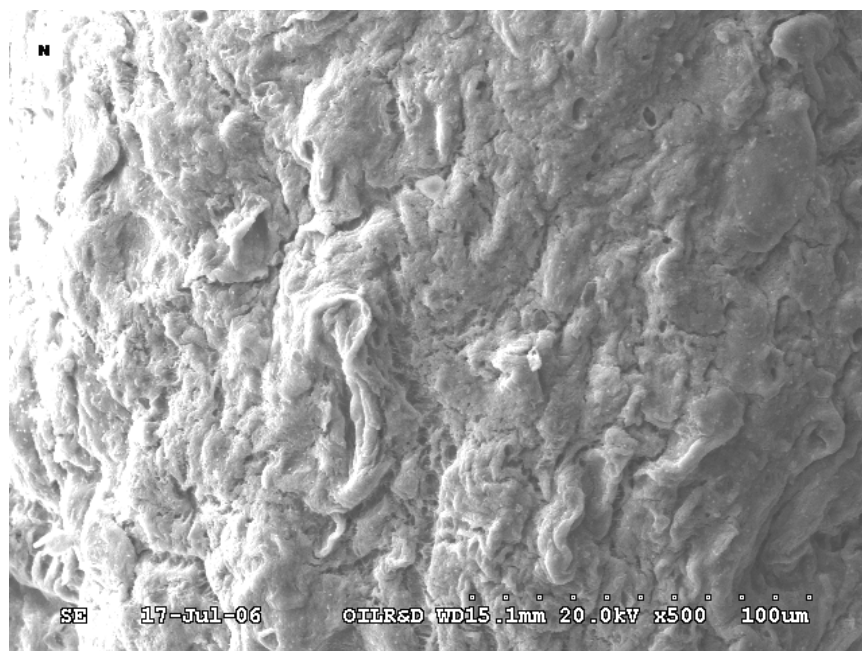


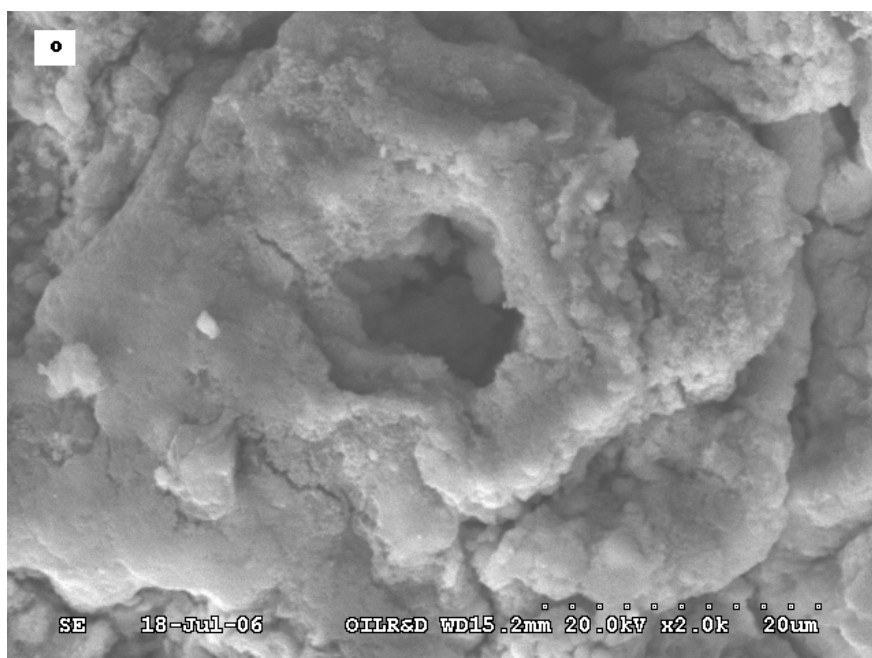


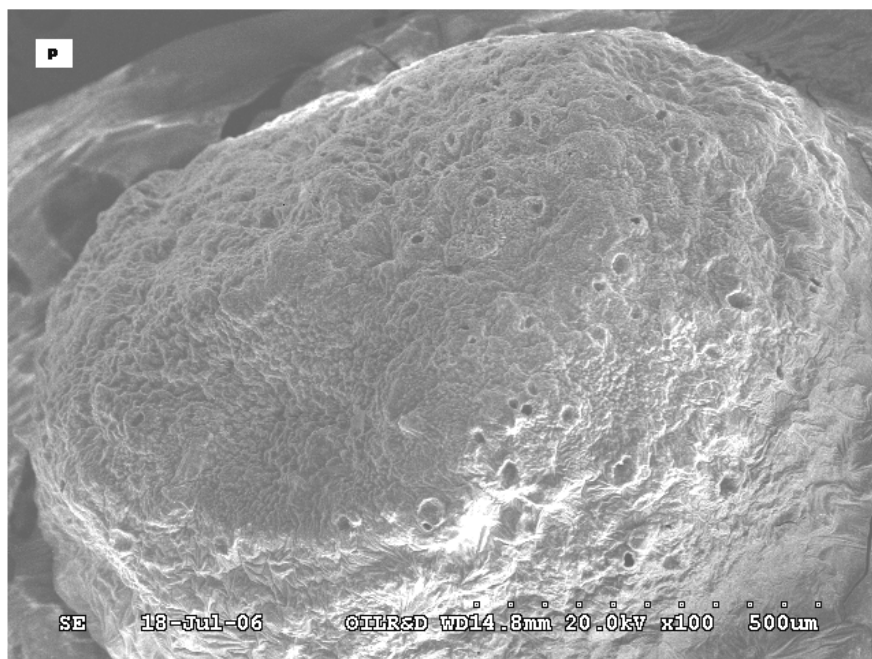
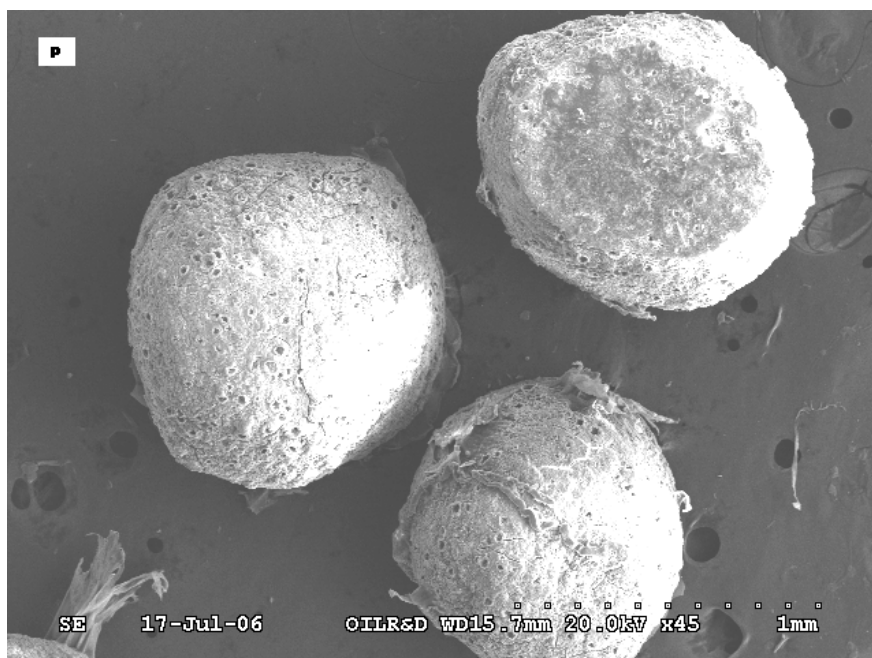












was taken as optimal and used throughout the study while evaluating the effect of other variables over the product characteristics. The prepared beads were observed under different magnifications to analyze the surface and morphology of the beads before and after dissolution. Cross-linking agent has shown a major effect on morphology. Beads cross-linked with BaCl_2 were smooth and non-porous. Whereas the $\text{Al}_2(\text{SO}_4)_3$ has produces very much irregular beads

and could not be successfully used as a cross-linking agent. The spherical beads by ionic-gelation method could not be produced with a ratio of *Bora rice* and sodium alginate below 2:1, because of the insufficient crosslinking and entanglement. But further increase in the sodium alginate concentration above 2:1 ratio has not shown added advantage. The scanning electron micrographs taken before and after dissolution in the three different dissolution mediums depict the effect of release environment. SEM photographs after the dissolution have shown the holes on to the surface of beads this explains the release of drug during dissolution that may be escaped out of beads forming these pores on to the surface.

The HPMC coating rendered the smooth surface to the beads. The coated surface in the coated Calcium cross-linked beads is clearly focused showing the HPMC film on to the spherical beads that remains after the dissolution. The pinholes are formed on the coated surface after the dissolution that witnesses the drug release during the dissolution from the beads. The formation of pores on the surface of the microbeads indicates that the drug release from the beads is possibly by the diffusion. The SEM photographs shown that the uncoated beads after dissolution in alkaline buffered medium has become highly porous and fluffy which revealed that the alkaline medium posed much stress on to the microcarrier system and induced the erosion.

CONCLUSIONS

The scanning electron microscopy revealed the size and surface characteristics of the microcarriers prepared using proposed polymeric backbone. The scanning electron micrographs of the different formulations prepared with varying process and formulation parameters depicted the effect of these parameters on size, shape, surface and mechanistic properties of the beads.

Acknowledgements

Authors are thankful to Oil India Limited, Duliajan for extending their research facilities to us for scanning electron microscopy of microbeads, and to Dr. George B Gilfellow for his kind help in procuring the same.

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