



## Synthesis and Characterization of Pectin Beads for the Smart Delivery of Agrochemicals

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### Authors' contributions

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aim:** The study was conducted to design pectin beads for achieving slow release of agrochemicals in wetlands via ion gelation method.

**Place and Duration of the Study:** The laboratory experiment was carried out at the Department of Nano Science & Technology, Tamil Nadu Agricultural University, Tamil Nadu during March-July 2021.

**Methodology:** Pectin beads were synthesized varying the concentrations of pectin (4, 6, 8, and 10 per cent) and calcium chloride (CaCl<sub>2</sub>) concentrations (0.5, 1, and 2 per cent). Calcium pectinate beads of different combinations were synthesized via ion gelation method. Calcium pectinate beads were characterized based on the recovery yield of beads, while surface characterization was done through Scanning electron microscope (SEM) and optical microscope to understand the topography of beads and assess the size of the beads respectively. Pore volume and surface area were also studied using BET (Brunauer-Emmett-Teller) analyzer.

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**Results:** The yield of calcium pectinate beads were higher while using the concentration of pectin @ 4 per cent and  $\text{CaCl}_2$  @ 2 per cent as cross-linking agent. The spherical and smooth surface of beads was achieved with the concentrations of 6 and 2 per cent pectin and  $\text{CaCl}_2$  respectively, while beads were flat and smooth with concentration of pectin @ 4 per cent. Similarly, complete solubility of pectin was not achieved with concentrations of 8 and 10 per cent. BET results of beads showed that beads are non-porous in nature.

**Conclusion:** Pectin and  $\text{CaCl}_2$  concentrations @ 6 and 2 per cent respectively were found to be ideal for the delivery of agrochemical based on the yield and surface morphology.

*Keywords: Pectin; encapsulation; agrochemicals; ion gelation method.*

## 1. INTRODUCTION

Agrochemicals are the natural and synthetic inputs that are applied to the crop for improving production, which comprises pesticides (fungicides, insecticides, herbicides, acaricide, nematocidal and bactericide) and fertilizers. Insect pests and diseases are the major biotic threat in the agricultural production system causing a yield loss of 45 per cent [1]. De et al., [2] reported that global consumption of pesticides was about 2 million tons per year, out of which the usage of herbicides (47.5%) rank first among the total pesticide consumption to manage weeds, followed by insecticides (29.5%), fungicides (17.5%) and other pesticides (5.5%). Herbicides undergo various degradation processes in the soil after application, which declines the herbicidal activity to control weeds. Degradation pathways include leaching, adsorption in organic matter and clays, photodecomposition, volatilization, microbial and chemical degradation. The fate of herbicides impacts the chemical efficiency and resulted in poor weed control efficiency. Moreover, intermediate products that resulted during the degradation contaminate the groundwater through leaching [3]. Therefore, encapsulation is an ideal strategy to minimize environmental contamination and maximize the use efficiency of agrochemicals. Various organic and inorganic polymers are used for encapsulation. Pectin is an organic polymer widely used as a carrier in the pharmaceutical field. However, the knowledge on the encapsulation of agrochemical in pectin is lacking. Hence, studies were designed to synthesize pectin beads using ion gelation method.

Pectin is a complex and linear polysaccharide that naturally exists in the higher plant's cell wall. It comprises chains of D-galacturonic acid units, which are linked by  $\alpha$ -(1-4) glycosidic linkage [4,5]. It is a biopolymer employed as a thickening agent, gelling agent, and stabilizer in the food

and beverage sector, while pectin is used for the delivery of drugs in the pharmaceutical fields [6]. Pectin is a cell wall polysaccharide and ideal biopolymer due to its excellent and unique characteristics viz., more stability at acidic conditions and high temperature, gelation property, non-toxicity, easy availability, biocompatibility, and low cost [7]. Molecular weight and degree of esterification and sources of pectin determine gelling ability. Similarly, pH, presence of solutes, number of side chains and arrangement (arabinan, galactan, or arabinogalactan units) and charge density governs the gelation of pectin [8]. Pectin is categorized into low and high methoxyl pectin based on the degree of esterification [5]. Low methoxyl pectin has less than 50% of methoxyl group content, while high methoxyl group pectin contains more than 50 per cent of the methoxyl group. Low and high methoxyl pectin have different gelling mechanisms, where high methoxyl pectin requires acid and sugar for the formation of gel and low methoxyl pectin forms gel complexing with divalent cations. Hydrogen bonding and hydrophobic interactions are responsible for gel formation in high methoxyl pectin [5]. Similarly, hydrogen bonding between free carboxyl groups and hydroxyl groups of neighboring pectin molecules also cause gelation in high methoxyl pectin. In contrast, ionic and electrostatic bonding are responsible for gel formation in low methoxyl pectin [4]. During gelation, cation bridges are established between carboxyl groups of low methoxyl pectin chains [8].

Commercial pectin is extracted from citrus peel and apple pomace which constitutes 20-30% and 10-15% of pectin available in the market respectively [6,9]. Pectin extracted from other sources is poor in gelling behavior [4]. The study used low methoxyl pectin for the formation of beads with calcium ion through ion gelation method.

## 2. MATERIALS AND METHODS

Pectin and calcium chloride anhydrous were procured from Himedia laboratories private limited for the synthesis of pectin beads. Ethanol (99.9%) was purchased locally for washing of beads, while deionized water was used for the synthesis of beads. Various concentrations of pectin (4, 6, 8, and 10 per cent) and calcium chloride (0.5, 1, and 2 per cent) were used to standardize for the formation of beads through ion gelation method. Pectin was dissolved in deionized water to obtain appropriate concentrations using a magnetic stirrer @ 400 rpm. Similarly, calcium chloride was dissolved with water separately using the magnetic stirrer for 60 minutes at 400 rpm. Pectin was extruded drop-wise @ 4 ml min<sup>-1</sup> through a syringe (0.55 mm) into calcium chloride solution maintaining the collection of a distance of 4-5 cm. Pectin beads were formed instantly in calcium chloride gelation solution. The beads were washed after curing for 30 minutes in the gelation bath with deionized water and ethanol. Then, beads were dried at room temperature to obtain constant weight in successive measurements. The beads were stored in an airtight container at room temperature for further characterization.

Calcium pectinate beads were weighed after drying, and chemical yield was determined by using the following formula [10]. The chemical yield is expressed in percentage.

$$\text{Chemical yield (\%)} = \frac{\text{Total mass of dried beads}}{\text{Total mass of raw material}} \times 100$$

The size of the beads was assessed through a optical microscope (Zeiss Axiolab 5) attached with a digital camera (Axiocam 208 color). Images were processed for measuring the diameter of the bead using Zen 3.0 (Black edition) and Image J Software. Calcium pectinate beads were sputter-coated for imaging in a scanning electron microscope (Quanta 250, FEI, Czech Republic) for surface characterization of beads. Images were captured at magnifications of 80x, 150x, and 500x at the voltage of 10kV. Brunauer-Emmett-Teller (Quantachrome NOVAtouch NT2LX-1) instrument was used to determine the average pore radius, surface area and pore volume of beads. Nitrogen gas was utilized as absorbate and samples were weighed before degassing process. Samples are heated to 100°C at ramp temperature of 10°C/min held for 3 hours for removing the moisture. Specific surface area of pectin beads was determined by using the multipoint BET method. Data were analyzed statistically using Agres software.

## 3. RESULTS AND DISCUSSION

Pectin and calcium chloride concentrations largely influenced the morphology and chemical yield of calcium pectinate beads. Higher pectin concentration decreased the chemical yield (Table 1). The chemical yield was observed to be increased with the concentration of calcium chloride concentrations. Pectin and calcium chloride concentrations @ 4 and 2 per cent respectively gave in a higher yield of calcium pectinate beads followed by the ionic gelation of 6 and 2 per cent of pectin and calcium chloride respectively. More cross-linking at higher concentration of calcium chloride were attributed to obtaining higher chemical yield.

Intermolecular crosslinking between the carboxyl groups of different pectin chains and divalent cation like calcium takes place due to polyanion-cation-polyanion interactions. Unbranched non-esterified galacturonan units in pectin involve in the complexing reactions with divalent calcium ions. Generally, gelation rate increases with the availability of more polymer substrate for binding with divalent cations during ion gelation process. Hence higher concentration of pectin and divalent calcium ions resulted in a higher chemical yield. Similarly, more cations in the reaction medium facilitate better surface and core cross-linking resulting in stable calcium pectinate beads. Cross-linking of pectin chains exhibits the egg-box model. A higher concentration of calcium chloride concentration produces strong and rigid gel [4,5]. A gradual increase in the chemical yield was observed with higher pectin concentration and however, the gradual decline in the chemical yield was recorded with pectin concentration of more than 6 per cent. Slow rearrangement of polymeric chains in higher pectin concentration for cross-linking with calcium ions resulted in poor gelation. Similarly higher viscosity of pectin at higher concentrations causes hindrance between polymeric chains resulting in poor gelation. Calcium pectinate beads with higher surface cross-linking aid higher encapsulation efficiency and achieve a slower release rate [11]. Spherical beads with a smooth surface of pectinate beads were observed with ion gelation of 6 and 2 per cent of pectin and calcium chloride respectively. Meanwhile, poor solubility of pectin at higher concentrations of 8 and 10 per cent resulted in the tail-like protrusion in the bead. The higher viscosity of pectin solution @ 10 per cent affected the roundness of beads resulting in oval-shaped beads [12].

Pectin beads obtained with various concentrations of pectin and calcium chloride are presented in Figs. 1-8. The shape and morphology of calcium beads is briefly described in Table 2. Viscosity and surface tension of pectin, concentration of calcium chloride in gelation bath, surface tension and viscosity of gelation bath, dripping diameter tip and collection distance during the ion gelation process affect the shape of the bead. Surface tension of pectin, the concentration of calcium chloride in bath, dripping diameter tip and curing time in gelation bath influence the size of calcium pectinate beads [13,14]. When pectin solution hits the surface of the gelation bath of calcium chloride solution, the bead is deformed at the gelation bath-air interface. The droplet is detached from the surface of the gelation bath (liquid-air interface) and deformed again due to the influence of surface tension and gelation process. The droplet regains the spherical shape after reaching the bottom of the gelation bath (Fig. 9). Generally low concentration of polymer

solution with less viscosity results in the formation of deformed flattened beads due to the collision of polymer solution with gelation bath. Similarly, viscous forces within the droplet and drag forces from the gelation medium influence the final shape of beads. Viscous forces in low polymer concentration exerts less force compared to the drag forces from gelation bath which disrupt the regain of the spherical shape of beads, while viscous force in the higher polymer concentration overcomes the drag force from the surrounding gelation bath and hence regain the spherical shape of beads. A similar trend was observed with increasing concentration of pectin concentration. Generally, calcium ions penetrate the core of polymer droplets resulting in the removal of water and the formation of rigid beads. The higher concentration of calcium ions in gelation bath results in the compact beads because of the expulsion of more water molecules from the droplet. Similarly higher calcium ions accelerate the gelation kinetics facilitating the stable beads.

**Table 1. Chemical yield of calcium pectinate beads at various concentrations of pectin and calcium chloride**

Treatments	Chemical yield (%)			Mean
	Calcium chloride at 0.5%	Calcium chloride at 1.0 %	Calcium chloride at 2.0 %	
Pectin concentration at 4%	62.50	73.25	89.50	75.08
Pectin concentration at 6%	77.34	77.11	79.90	78.12
Pectin concentration at 8%	51.88	65.88	73.75	63.84
Pectin concentration at 10%	55.60	54.60	66.30	58.83
Mean	61.83	67.71	77.36	

**Table 2. Shape and morphology of calcium pectinate beads at various concentrations of pectin and calcium chloride**

Pectin (%)	Calcium chloride (%)	Remarks
4	0.5	Beads were thin, flat and irregular in structure
	1	Flattened beads were observed without spherical shape
	2	Beads were found to be flat with round shape
6	0.5	Flattened and thin beads were obtained with oblate shape
	1	Beads were well-formed with poor regain of spherical shape
	2	Spherical beads were formed with smooth surface
8	0.5	Flat beads were obtained failing to regain spherical shape
	1	Beads were formed to be flat with an oblate shape
	2	Pear shaped beads were formed with smooth surface
10	0.5	Egg-shaped beads were obtained with little protrusion
	1	Tear shaped beads were formed
	2	Tear shaped beads were formed with a smooth surface

Generally, calcium chloride concentration in the gelation bath determines the size of the bead. Higher concentration of calcium ions results in higher gelation and tightening of the gel network structure leading to smaller-sized beads. This was evident in the experiment that a higher concentration of calcium chloride results in the small-sized beads. The mean diameter of beads with various concentrations of pectin and calcium chloride is given in Table 3. The concentration of calcium chloride at 0.5 per cent resulted in the larger-sized beads (1.1 mm), while a higher

concentration of 1 and 2 per cent produced smaller-sized beads (1.06 and 0.92 mm).

Concentration of pectin also affected the size of the bead, where a higher concentration of pectin decreases the size of the microspheres. Increasing the concentration of polymer solution increases the viscosity of the polymer solution exponentially. The increased viscosity of polymer solution results in the reduction of surface tension, which in turn reduced the size of the beads.

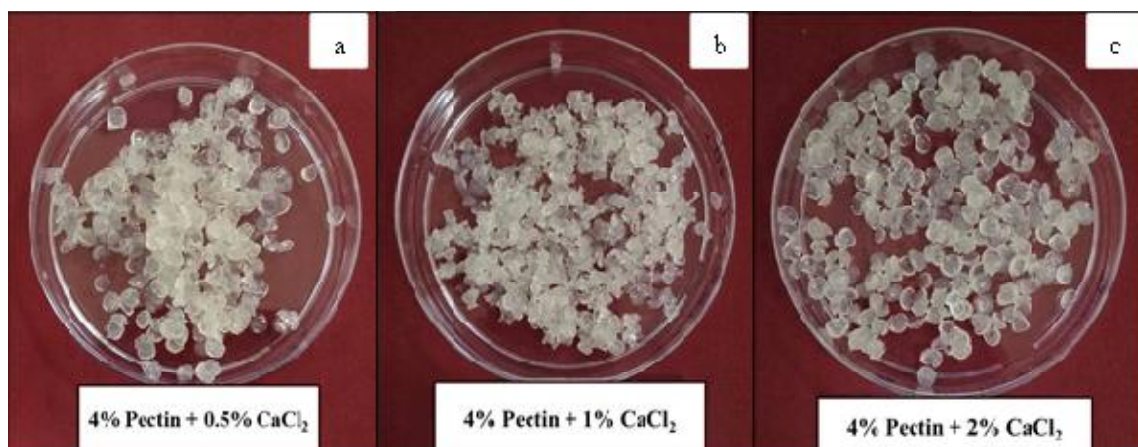


Fig. 1. 4% Calcium pectinate beads: a. 0.5% CaCl<sub>2</sub>, b. 1% CaCl<sub>2</sub> and c. 2%CaCl<sub>2</sub>

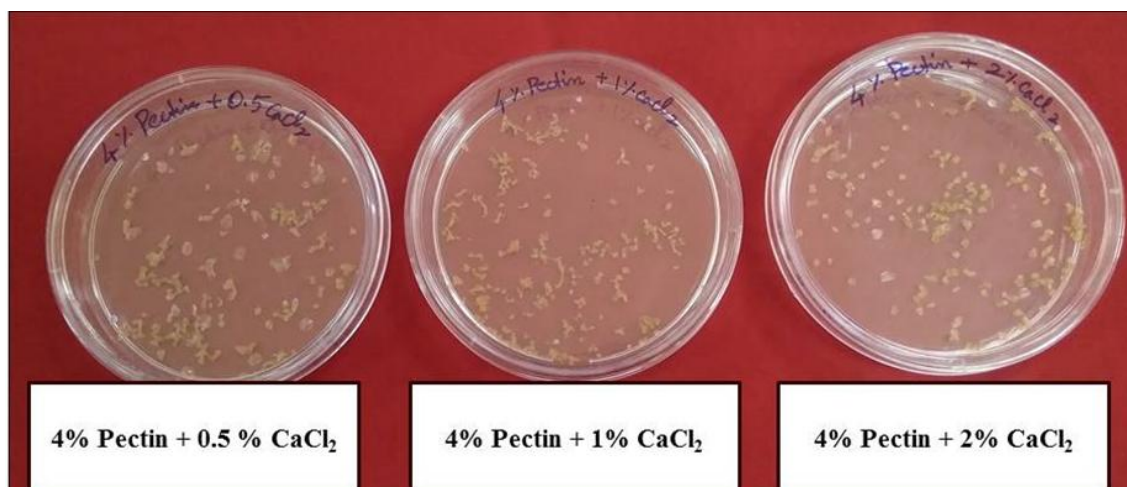


Fig. 2. 4% Calcium pectinate beads (after drying)

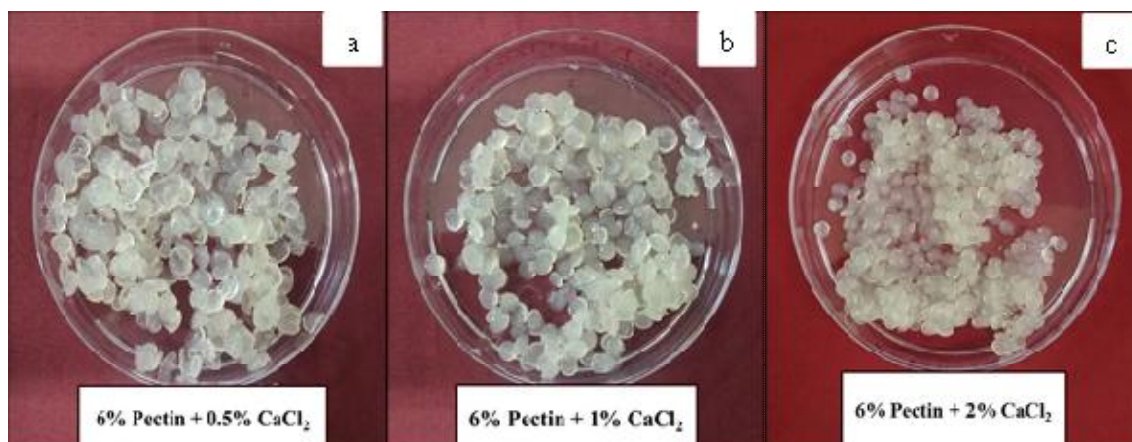


Fig. 3. 6% Calcium pectinate beads: a. 0.5% CaCl<sub>2</sub>, b. 1% CaCl<sub>2</sub> and c. 2%CaCl<sub>2</sub>

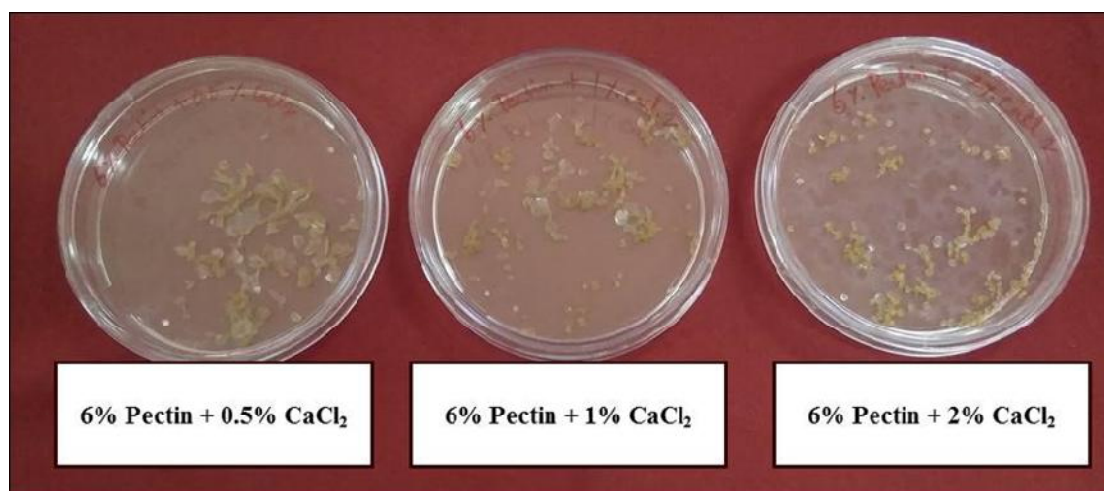


Fig. 4. 6% Calcium pectinate beads (after drying)

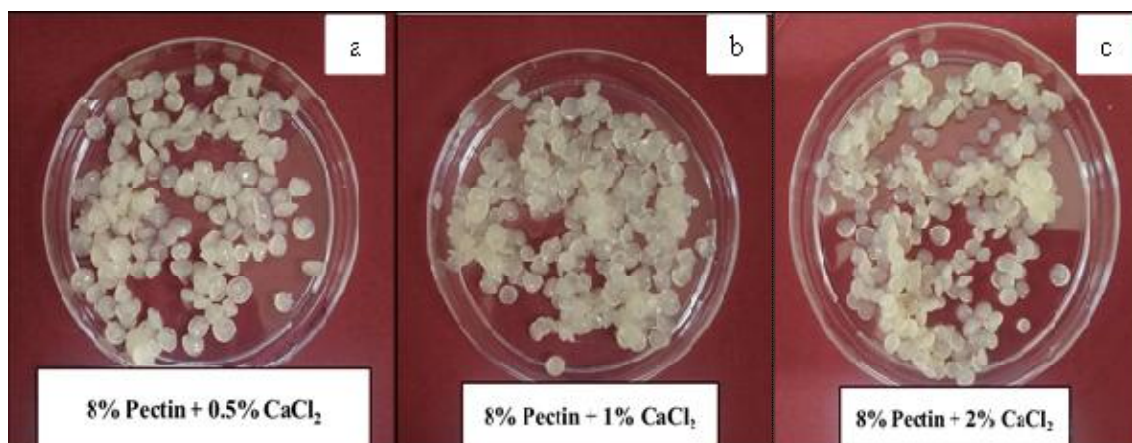


Fig. 5. 8% Calcium pectinate beads: a. 0.5% CaCl<sub>2</sub>, b. 1% CaCl<sub>2</sub> and c. 2%CaCl<sub>2</sub>

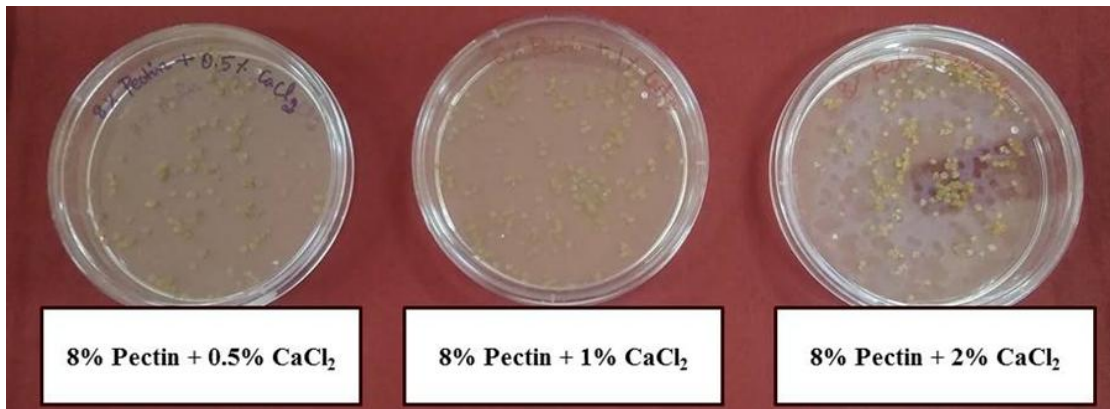


Fig. 6. 8% Calcium pectinate beads (after drying)

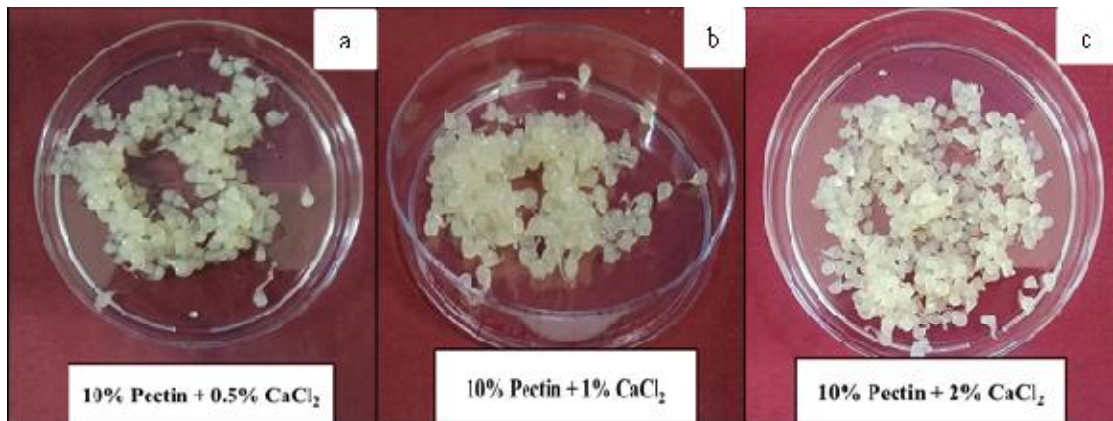


Fig. 7. 10% Calcium pectinate beads: a. 0.5% CaCl<sub>2</sub>, b. 1% CaCl<sub>2</sub> and c. 2%CaCl<sub>2</sub>

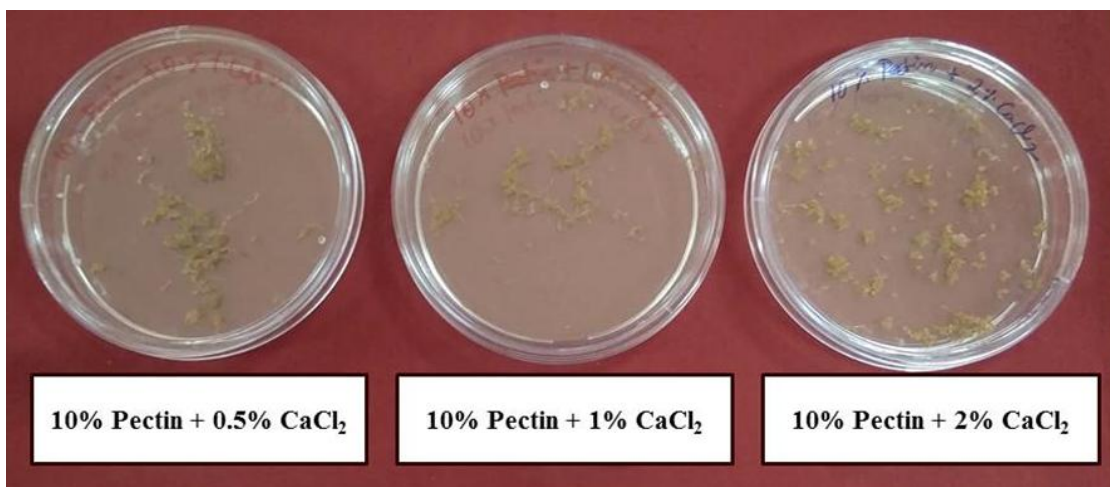
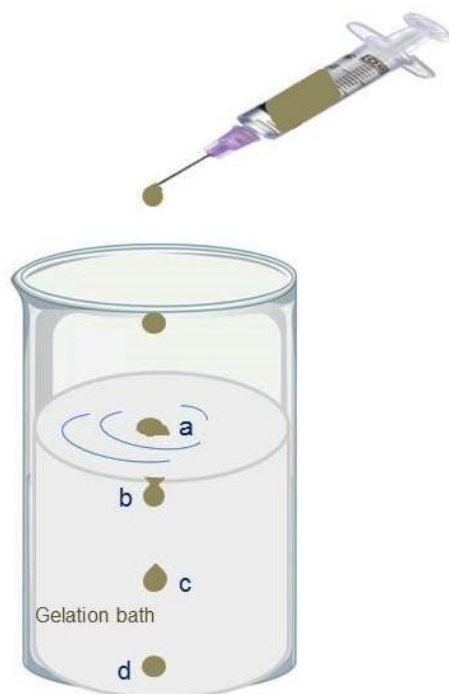


Fig. 8. 10% Calcium pectinate beads (after drying)



- a. Deformation of beads due to the impact of surface of gelation bath
- b. Detachment from the surface of gelation bath due to gel formation
- c. Deformation of beads in the gelation due to the drag forces from the surrounding medium
- d. Regain of spherical shape

**Fig. 9. Stages of bead formation in gelation bath**

Scanning electron microscopy imaging of calcium pectinate beads was carried out to assess the topography of beads (Figs. 10-21), where cracks over the bead were found at all concentrations of pectin. Cracks in pectin beads were measured which ranged between 2-11  $\mu\text{m}$ . Pectin beads were dried in Lyophilizer to obtain the constant weight of beads. Moreover, the isotherm of pectin beads followed type II isotherm [15]. Pore radius, pore volume (Tables 4 and 5) and isotherm (Figs. 22-33) of pectin

beads confirmed that pectin beads were non-porous. The fluctuation in isotherm at varying pectin concentration is due to the presence of cracks and reflected more with an increase in crack size. Similarly, surface area (Table 6) indicated that pectin beads were of micro particles. Moreover, pectin beads are matrix complexes ensuring the entrapment of active ingredients in the surface and inner core of beads.

**Table 3. Diameter of pectin beads**

Treatments	Bead diameter (mm)		
	Calcium chloride at 0.5%	Calcium chloride at 1.0 %	Calcium chloride at 2.0 %
Pectin concentration at 4%	0.90	0.94	0.95
Pectin concentration at 6%	1.14	0.99	0.93
Pectin concentration at 8%	1.44	1.13	1.11
Pectin concentration at 10%	0.79	0.77	0.74

\*SE(d)=0.09 CD(P=0.05)=0.19

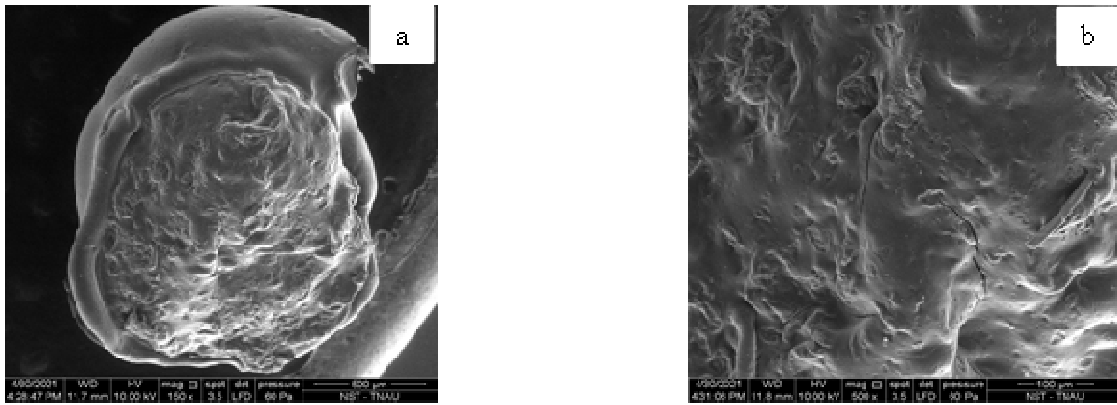


Fig. 10. SEM image of 4% Pectin+0.5% CaCl<sub>2</sub>: a. 150x and b: 500x

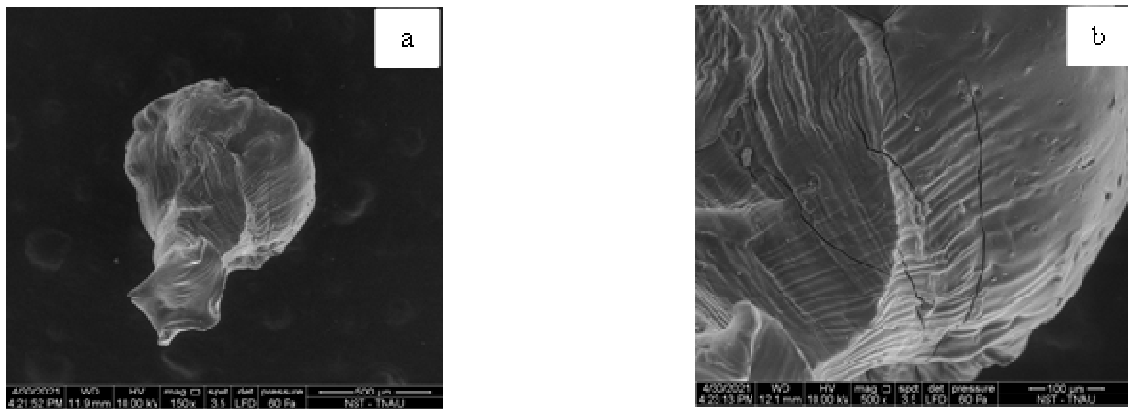


Fig. 11. SEM image of 4% Pectin+1% CaCl<sub>2</sub>: a. 150x and b: 500x

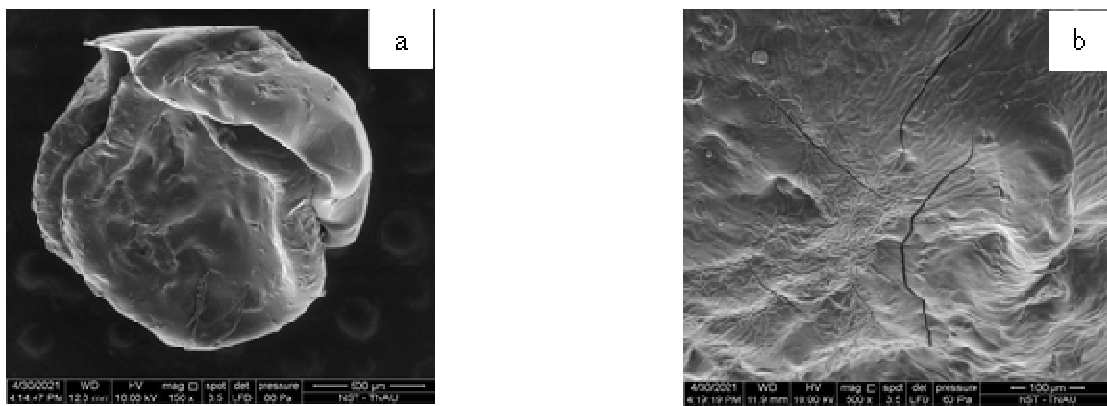


Fig. 12. SEM image of 4% Pectin+2% CaCl<sub>2</sub>: a. 150x and b: 500x

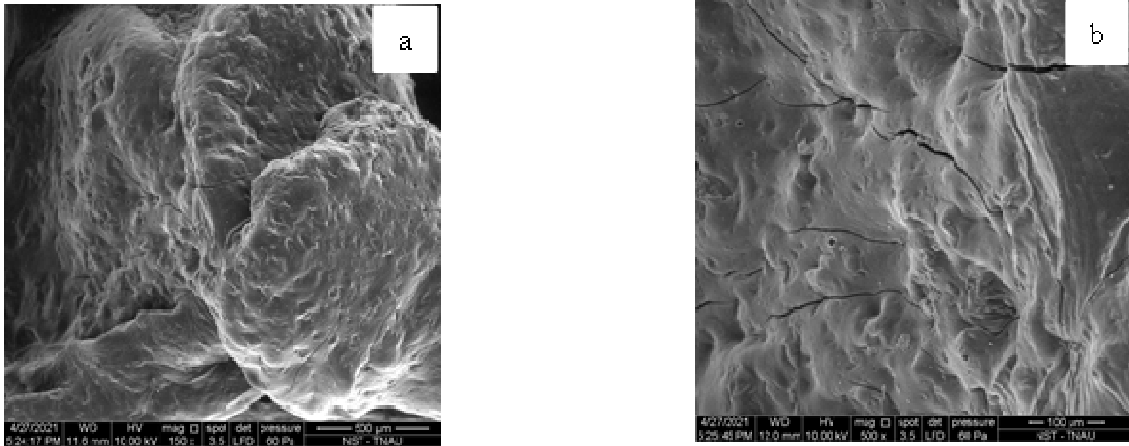


Fig. 13. SEM image of 6% Pectin+0.5% CaCl<sub>2</sub>: a. 150x and b: 500x

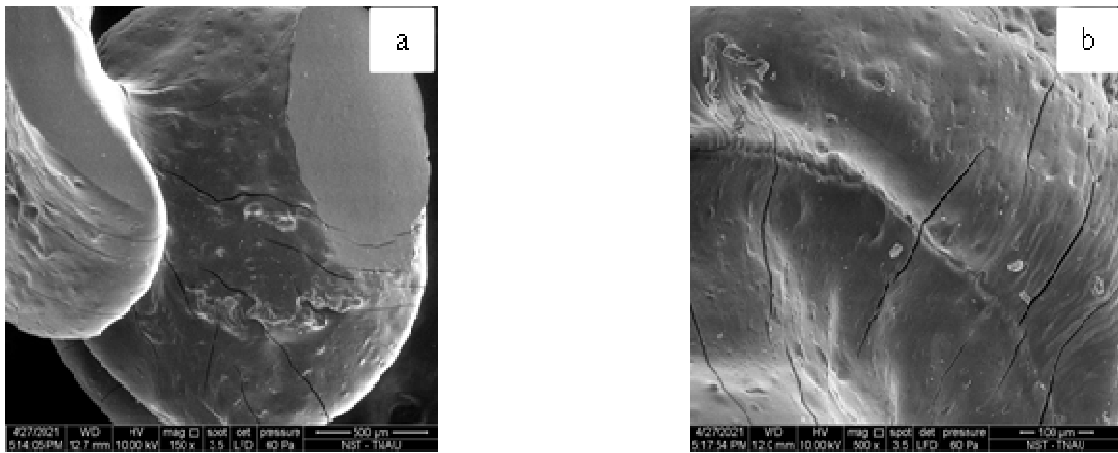


Fig. 14. SEM image of 6% Pectin+1% CaCl<sub>2</sub>: a. 150x and b: 500x

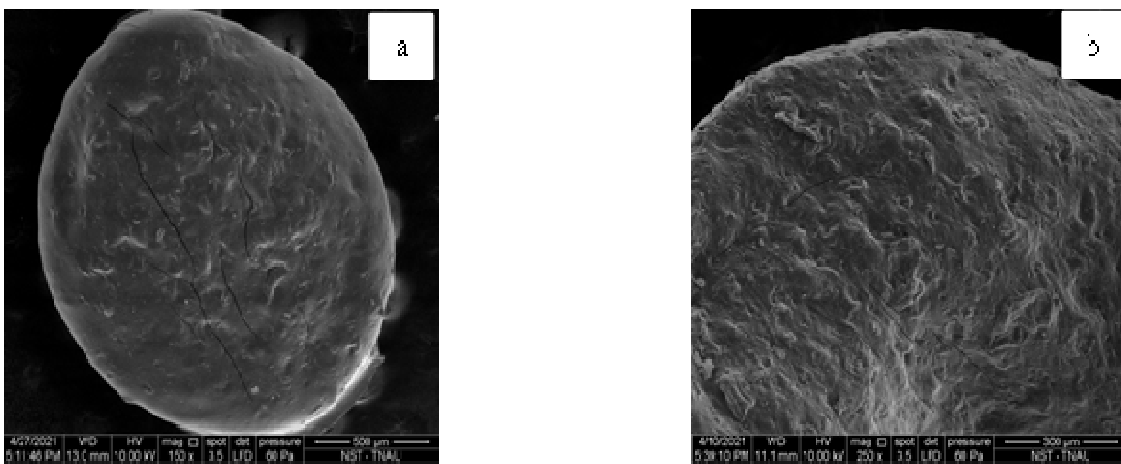


Fig. 15. SEM image of 6% Pectin+2% CaCl<sub>2</sub>: a. 150x and b: 500x

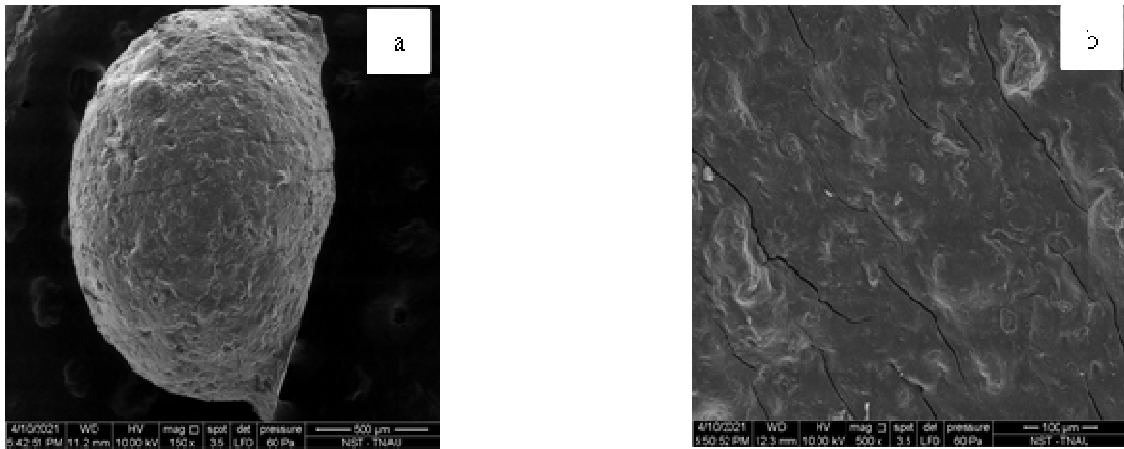


Fig. 16. SEM image of 8% Pectin+0.5% CaCl<sub>2</sub>: a. 150x and b: 500x

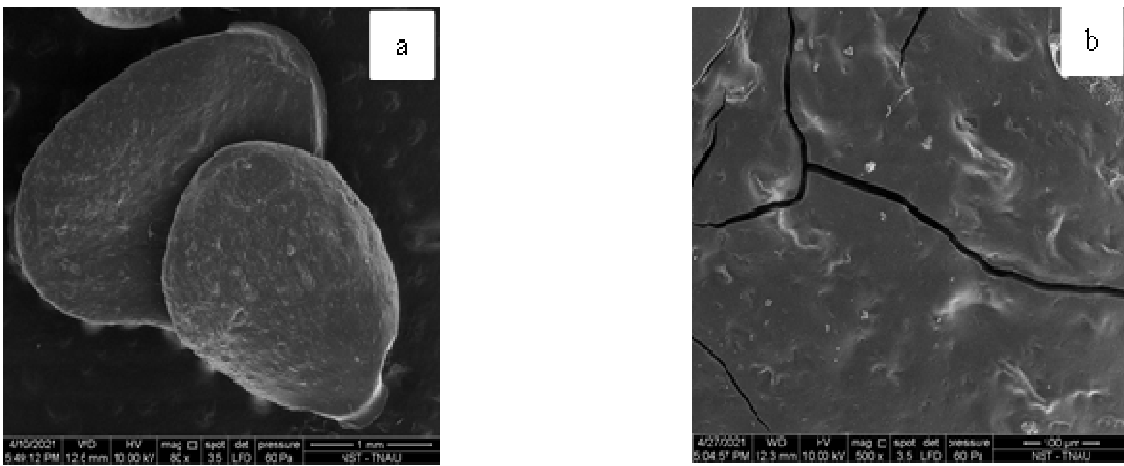


Fig. 17. SEM image of 8% Pectin+1% CaCl<sub>2</sub>: a. 150x and b: 500x

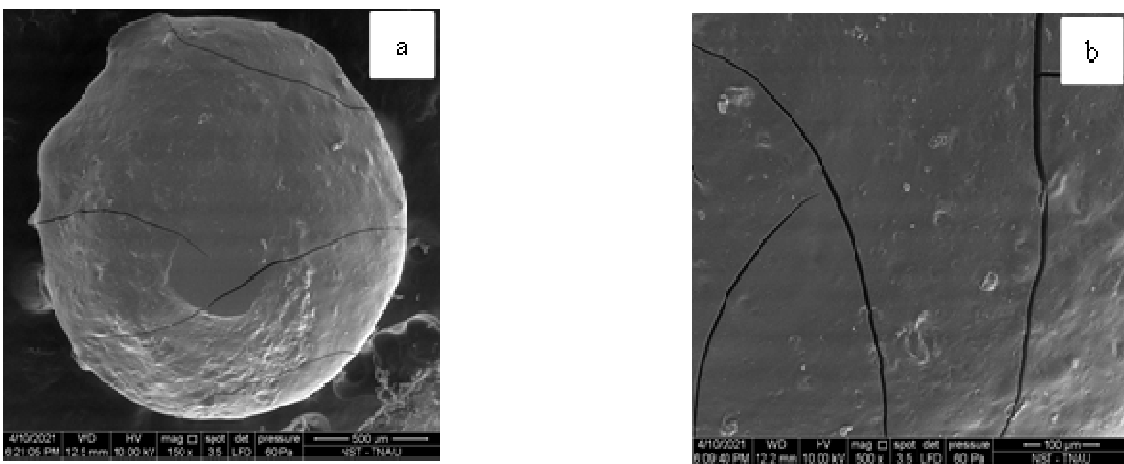


Fig. 18. SEM image of 8% Pectin+2% CaCl<sub>2</sub>: a. 150x and b: 500x

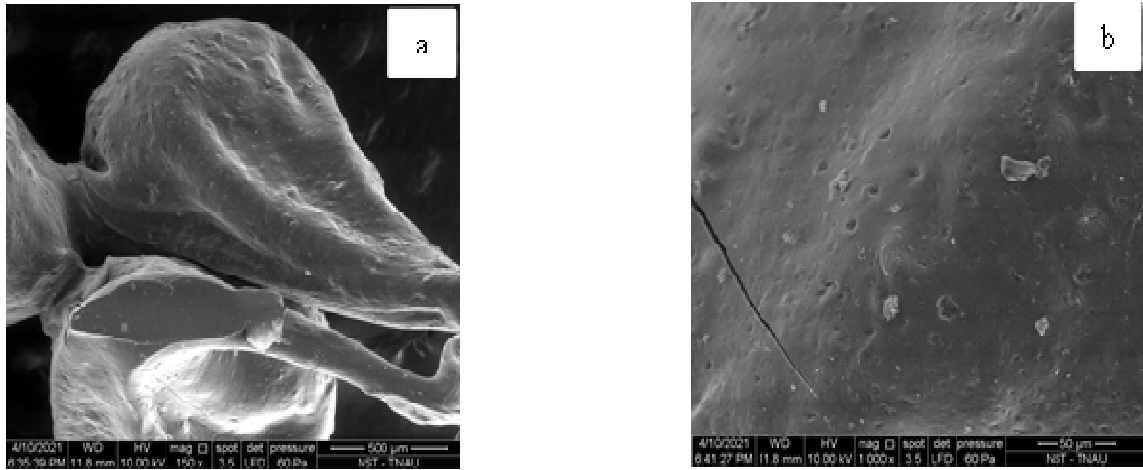


Fig. 19. SEM image of 10% Pectin+0.5% CaCl<sub>2</sub>: a. 150x and b: 500x

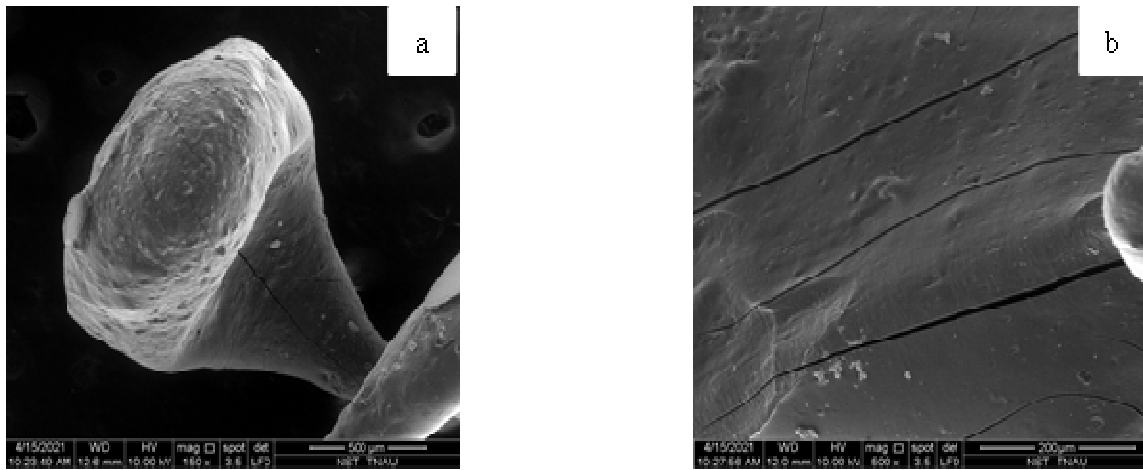


Fig. 20. SEM image of 10% Pectin+1% CaCl<sub>2</sub>: a. 150x and b: 500x

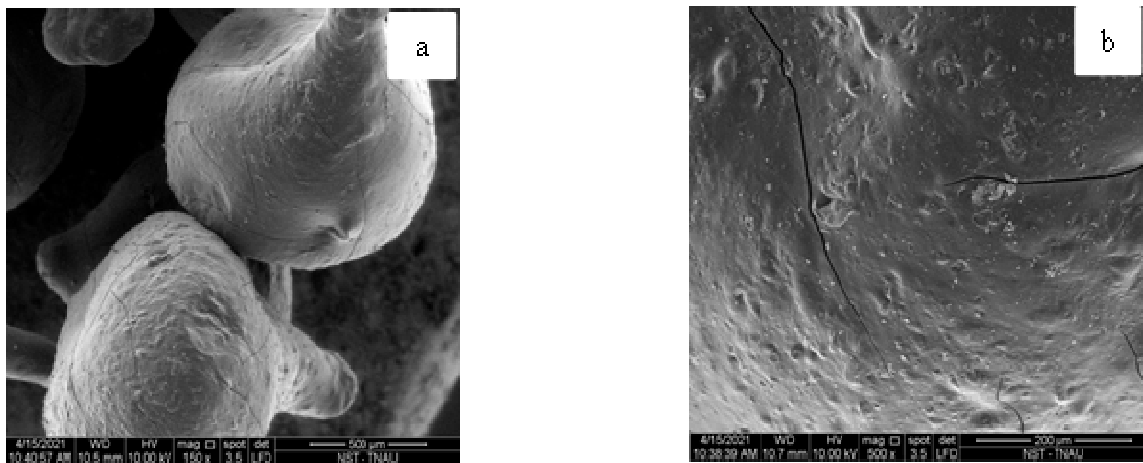


Fig. 21. SEM image of 10% Pectin+2% CaCl<sub>2</sub>: a. 150x and b: 500x

**Table 4. Pore radius of pectin beads obtained with different concentrations of pectin and calcium chloride**

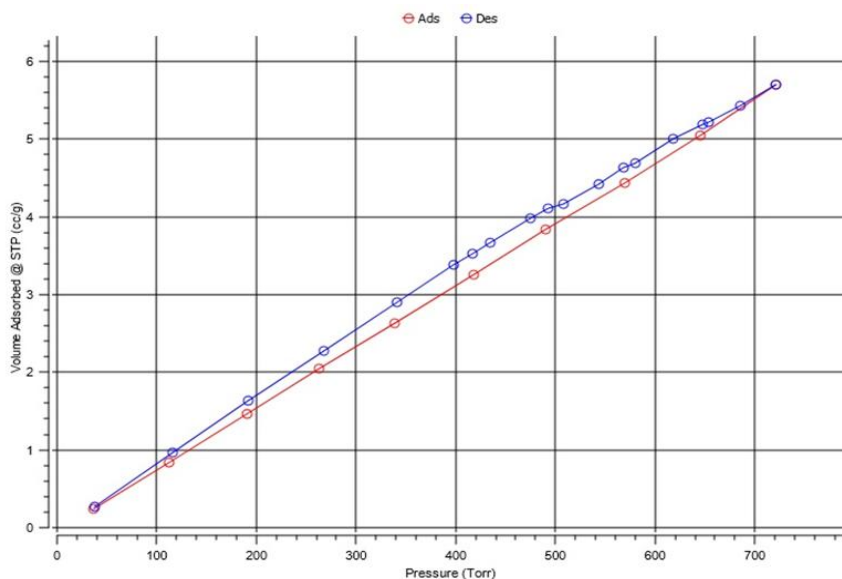
Treatments	Pore radius (nm)		
	Calcium chloride at 0.5%	Calcium chloride at 1.0 %	Calcium chloride at 2.0 %
Pectin concentration at 4%	1.2811	1.2370	1.0782
Pectin concentration at 6%	1.5601	-1.0429	1.3016
Pectin concentration at 8%	1.0118	1.7317	1.0904
Pectin concentration at 10%	1.1524	-1.6900	0.9149

**Table 5. Pore volume of pectin beads obtained with different concentrations of pectin and calcium chloride**

Treatments	Pore Volume (cc/g)		
	Calcium chloride at 0.5%	Calcium chloride at 1.0 %	Calcium chloride at 2.0 %
Pectin concentration at 4%	0.0085	0.0244	0.0345
Pectin concentration at 6%	0.0671	0.0177	0.0475
Pectin concentration at 8%	0.0476	0.1181	0.0110
Pectin concentration at 10%	0.0551	0.0135	0.0069

**Table 6. Surface area of pectin beads obtained with different concentrations of pectin and calcium chloride**

Treatments	Surface area (m <sup>2</sup> /g)		
	Calcium chloride at 0.5%	Calcium chloride at 1.0%	Calcium chloride at 2.0%
Pectin concentration at 4%	0.641	1.220	2.867
Pectin concentration at 6%	4.130	1.036	4.042
Pectin concentration at 8%	3.829	8.126	0.839
Pectin concentration at 10%	4.383	0.935	0.551



**Fig. 22. Isotherm curve of beads @ 4% Pectin+0.5% CaCl<sub>2</sub>**

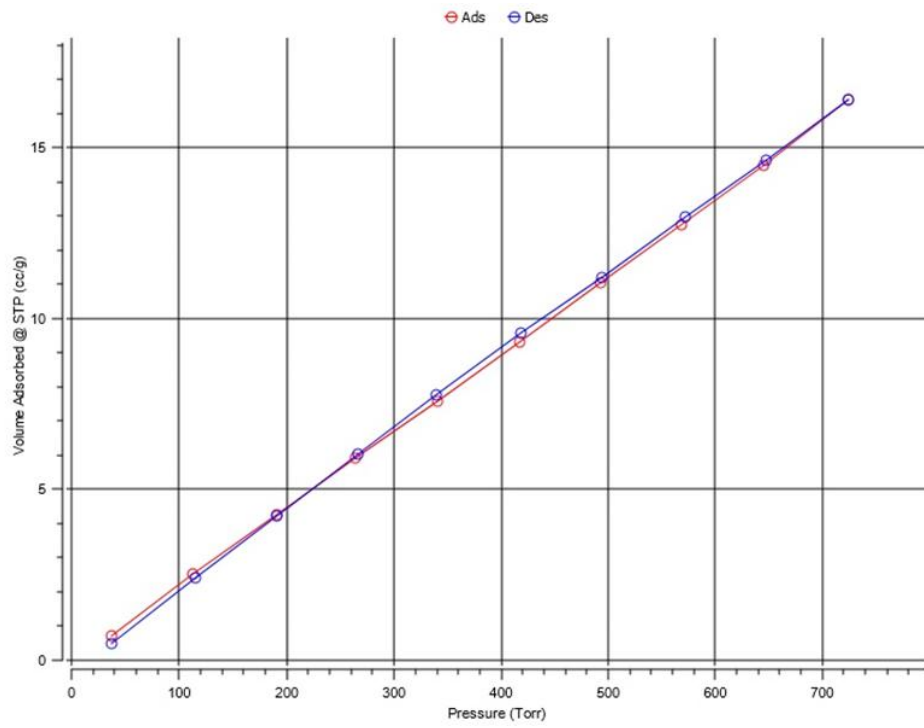


Fig. 23. Isotherm curve of beads @ 4% Pectin+1% CaCl<sub>2</sub>

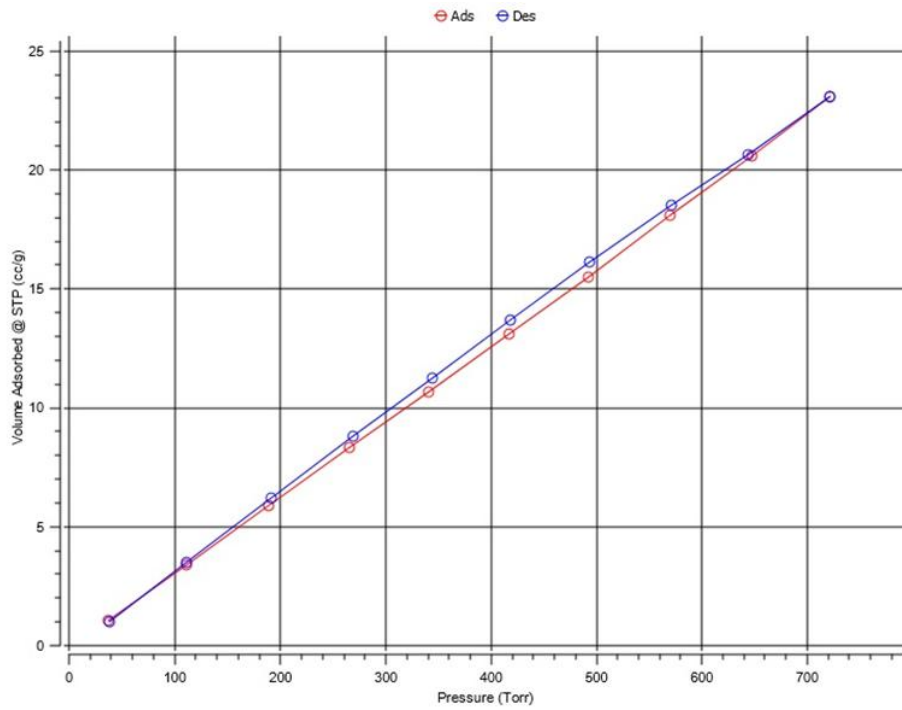


Fig. 24. Isotherm curve of beads @ 4% Pectin+2% CaCl<sub>2</sub>

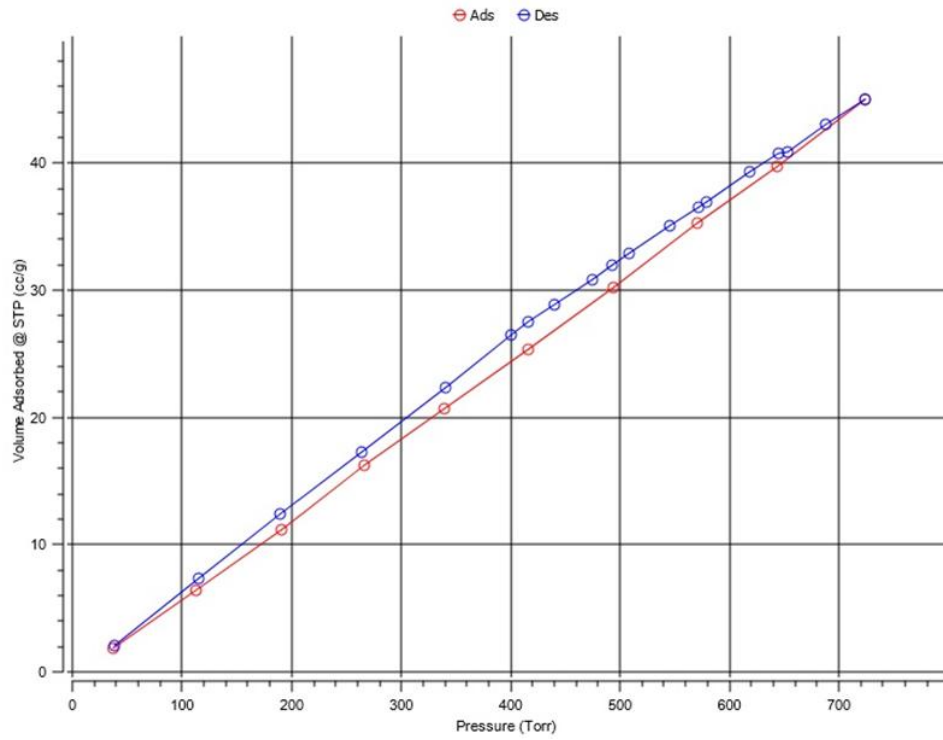


Fig. 25. Isotherm curve of beads @ 6% Pectin+0.5% CaCl<sub>2</sub>

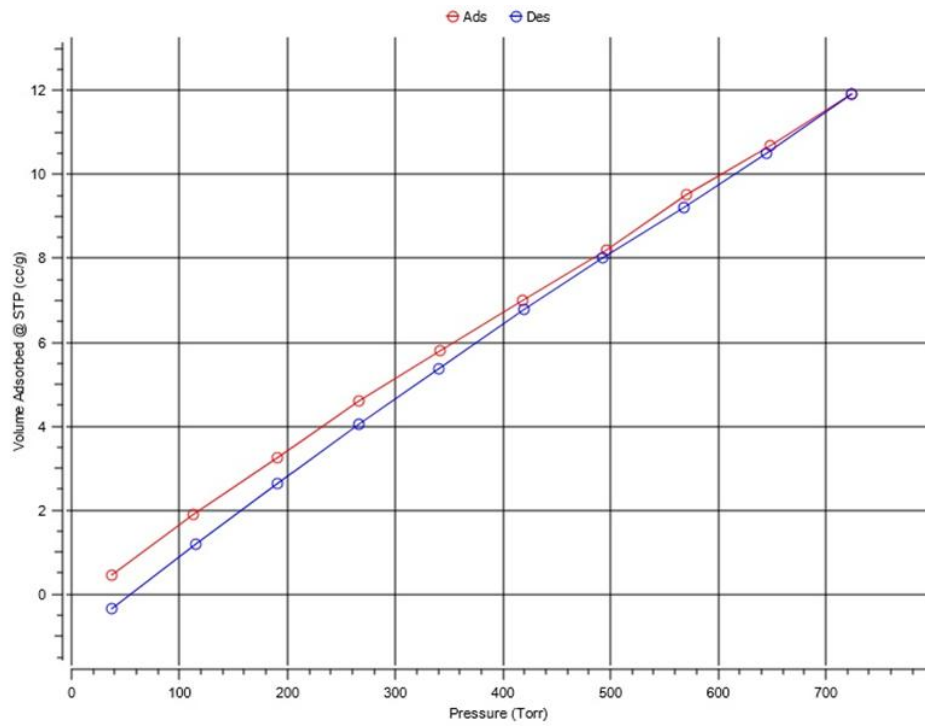


Fig. 26. Isotherm curve of beads @ 6% Pectin+1% CaCl<sub>2</sub>

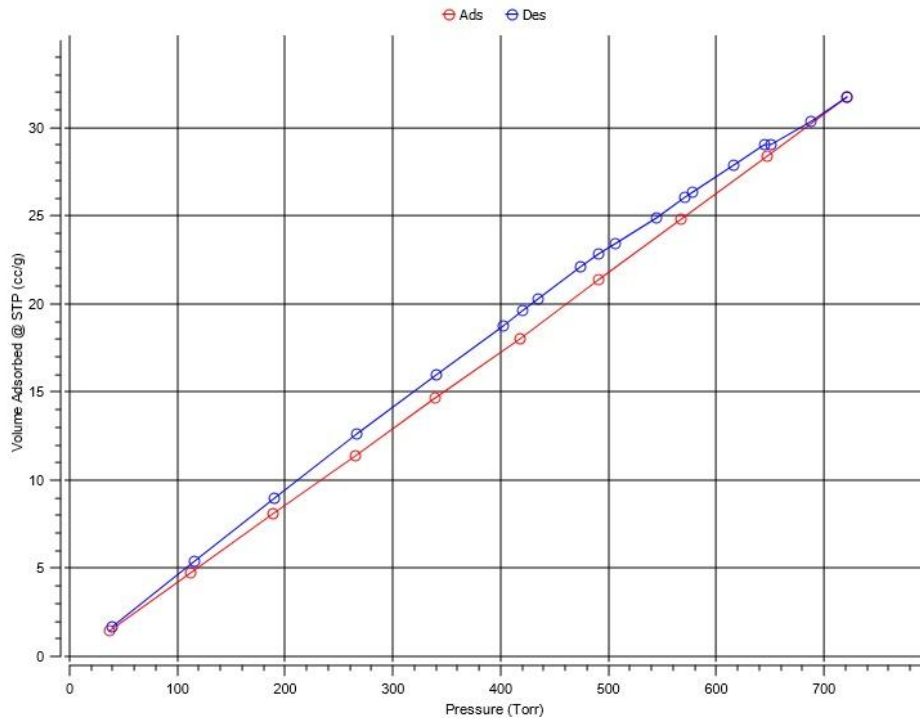


Fig. 27. Isotherm curve of beads @ 6% Pectin+2% CaCl<sub>2</sub>

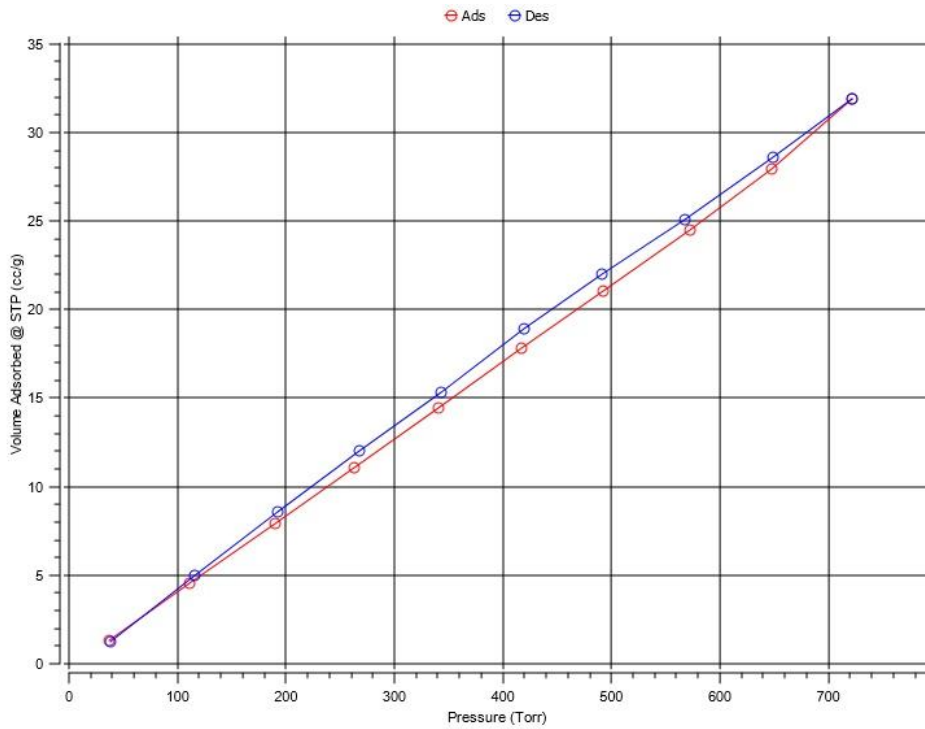


Fig. 28. Isotherm curve of beads @ 8% Pectin+0.5% CaCl<sub>2</sub>

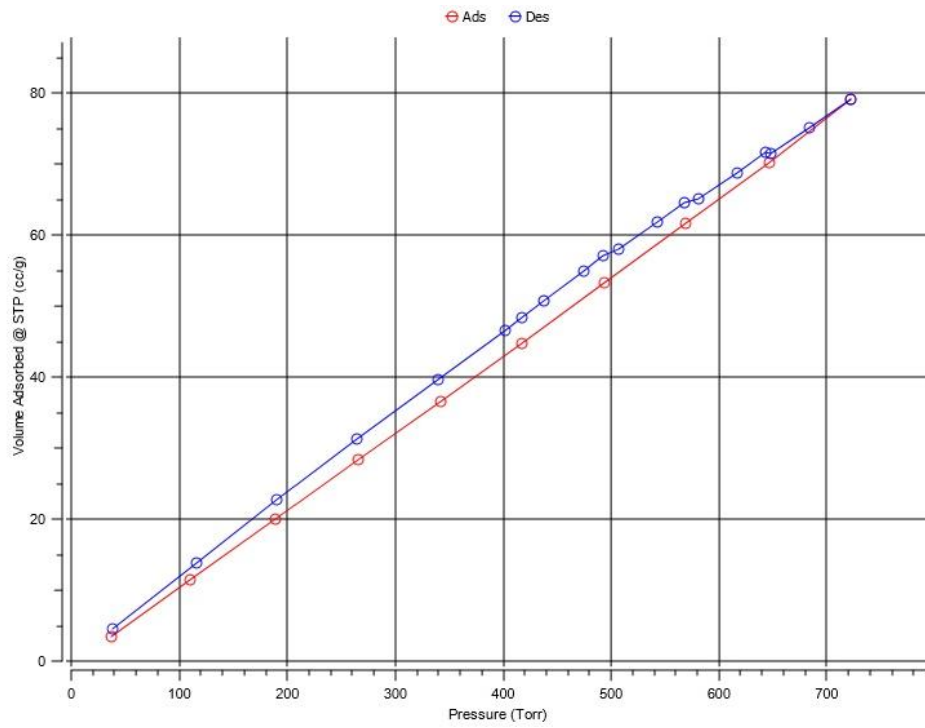


Fig. 29. Isotherm curve of beads @ 8% Pectin+1% CaCl<sub>2</sub>

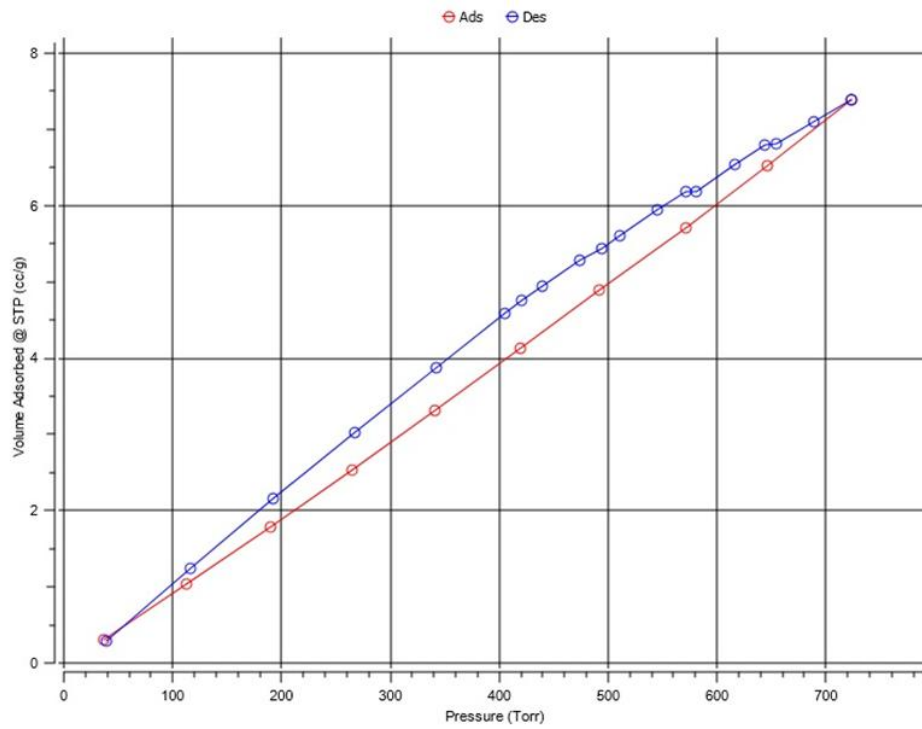
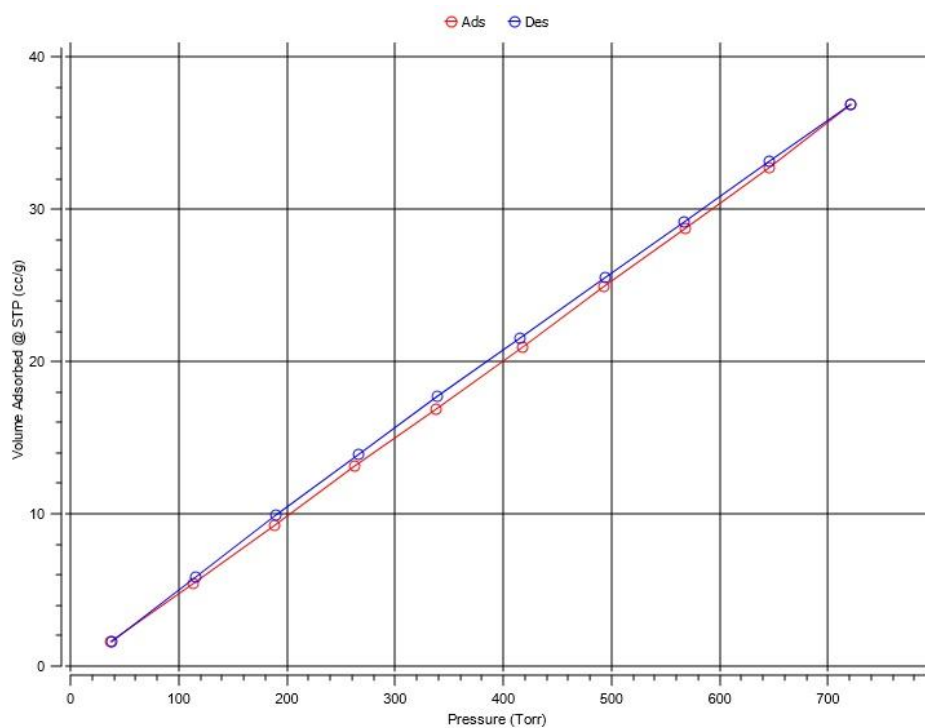
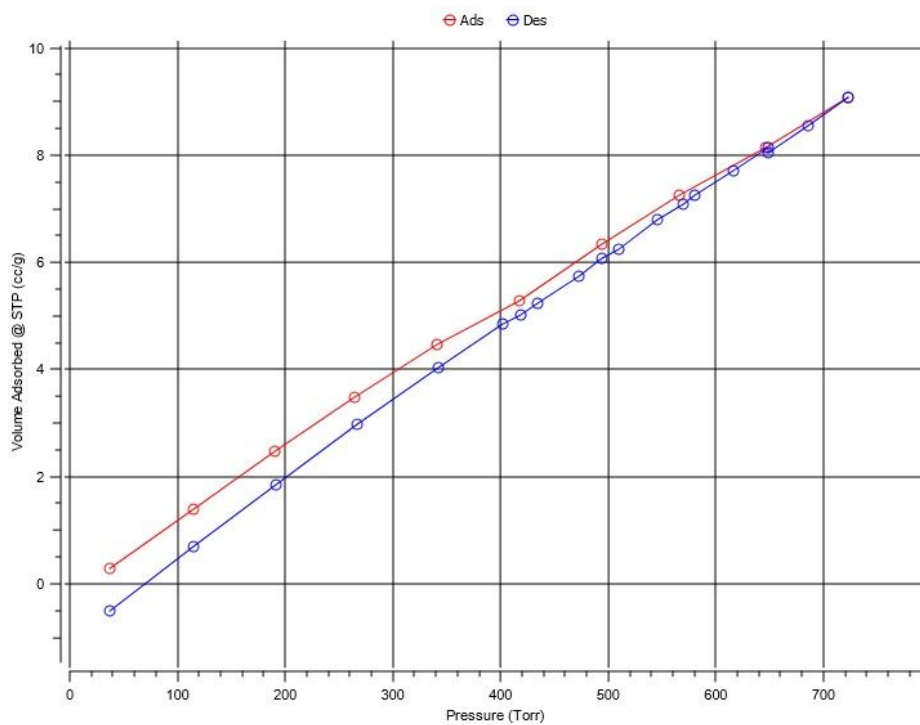


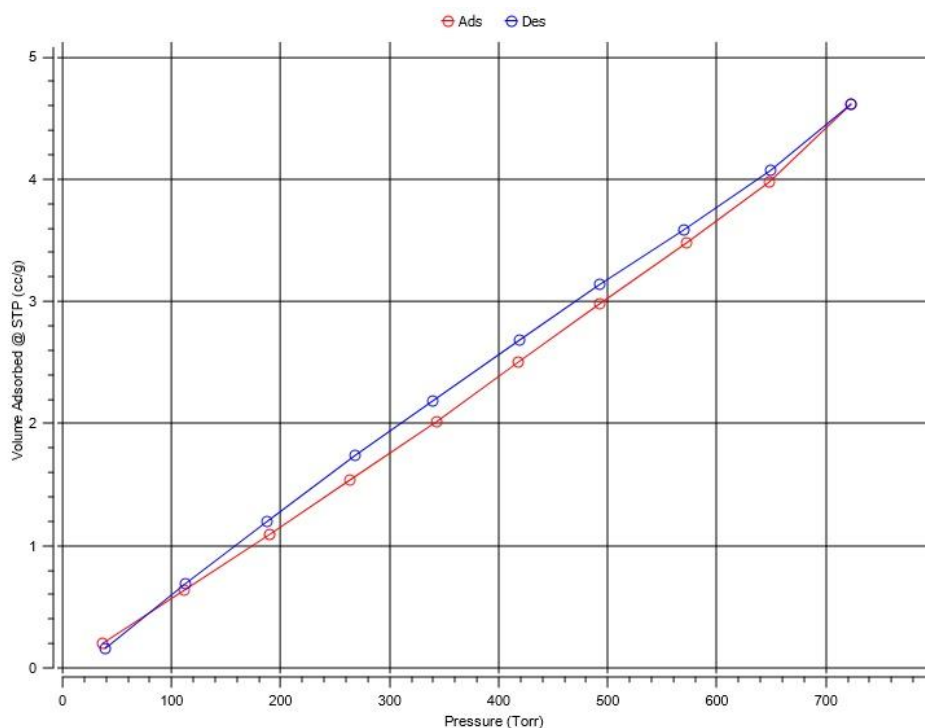
Fig. 30. Isotherm curve of beads @ 8% Pectin+2% CaCl<sub>2</sub>



**Fig. 31. Isotherm curve of beads @ 10% Pectin+0.5% CaCl<sub>2</sub>**



**Fig. 32. Isotherm curve of beads @ 10% Pectin+1% CaCl<sub>2</sub>**



**Fig. 33. Isotherm curve of beads @ 10% Pectin+2% CaCl<sub>2</sub>**

#### 4. CONCLUSION

The process factors affect the size and shape of calcium pectinate beads. Concentration of pectin and gelation medium are the main factors, which influenced the size, shape and stability of beads. Generally higher concentrations of polymer and gelation bath reduce the size of the bead, while lower and higher concentrations of pectin result in flattened and irregular beads respectively. The data on pore volume as well as the isotherm curve indicated the non-porous nature of beads. Beads made with pectin and calcium chloride of 6 and 2 per cent exhibited spherical with a smooth surface. Calcium pectinate beads with higher surface cross-linking provide an opportunity for achieving higher encapsulation efficiency of active molecules with a slower release rate.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for

any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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