Preparation of Chitin-Glucan Microsphere via Spray-Drying Technique and their Antibacterial Activity

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ABSTRACT

The experiment was designed to examine the microsphere of the chitin-glucan complex. We formed a chitin-glucan microsphere (ChGMS) from the spray dryer technique. SEM images observed that shape of ChGMS was spherical. From particle size analyzer and SEM analysis both showed that the size of particles was in the range of 1.5 to 3.5 µm. It showed amorphous nature after the formation of microsphere particles of chitin-glucan. The effect of chitin-glucan complex and ciprofloxacin loaded chitin-glucan microsphere on Bacillus subtilis *and* Escherichia coli *were also tested. Antibacterial analysis was indicating that the ciprofloxacin loaded chitin-glucan microsphere strongly inhibited the growth of bacteria in comparison to the chitin-glucan solution. From the analysis, we can conclude that the antibacterial property was also dependent on the size of the chitin-glucan microsphere. Complexation of the chitin-glucan microsphere with ciprofloxacin improved the antibacterial activity of the chitin-glucan microsphere.*

KEYWORDS: *Chitin-glucan microsphere, Antibacterial activity, Ciprofloxacin.*

INTRODUCTION

Chitin-glucan complex (ChGC) is a part of fungal cell walls linked with a biodegradable chitin (N-acetyl D-glucosamine) and glucan (poly-1, 4-D glucan). ChGC is a biodegradable, natural source cell wall of many yeast and fungi such as *Schizophyllum com-mune*[1], *Agaricus bisporus*[2], *Pichia pastoris*[3], *Aspergillus niger*[4], and also many other fungi. ChGC is natural, water-insoluble, non-toxic, biocompatible, and biodegradable polymer [5,6]. Therefore, ChGC has been used in biomedical areas such as antibacterial, anticancer, anti-inflammatory properties [7-9].

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In a previous work chitin-glucan complex has been isolated from *Agaricus bisporus* [2] and the size of the chitin-glucan complex is very large therefore, in this work, we have improved the size of the chitin-glucan complex by spray drying technique [10,11]. It is a single-step process that is solution was transformed into a fine powder and also it involves droplet formation. In this technique, we have prepared micro and nano particles, widely used in food and pharmaceutical industries. The properties of spray dryers are affected by many factors such as varies different concentrations of samples, inlet temperature, and feed pump. This technology is very low cost and easy to develop. This route is flexible, adjustable to normally use for processing equipment, and generate particles that give good properties.[12]

Ciprofloxacin is an antibiotic used for bacterial infection and belongs to a class of drugs namely quinolone antibiotics.[13-17] Herein, we have loaded ciprofloxacin in the chitin-glucan microsphere and to increase the antibacterial activity of the chitin-glucan complex.

In a recent report, Tao et al., 2013 [18] was prepared chitosan microsphere having inlet temperature was set at 160°C and the mean size of chitosan was measure around 4.07 µm. The size of the chitosan is large in comparison to our present work. In the chitin-glucan experiment mean size of the microsphere is below 4.07 µm. So, we can say that it is useful for many applications and also we can say that it is better for the chitosan microsphere.

In this work, we have determined the microsphere particles from the chitin-glucan complex with the help of a spray drying technique. Then ciprofloxacin was loaded in chitin-glucan microsphere for the improvement of antibacterial activity. Therefore, we have seen that ciprofloxacin loaded chitin-glucan microsphere give better result in comparison to chitin-glucan complex. Therefore we can conclude that decrease the size of the chitin-glucan complex increases the antibacterial activity.

EXPERIMENTAL

Materials & Methods

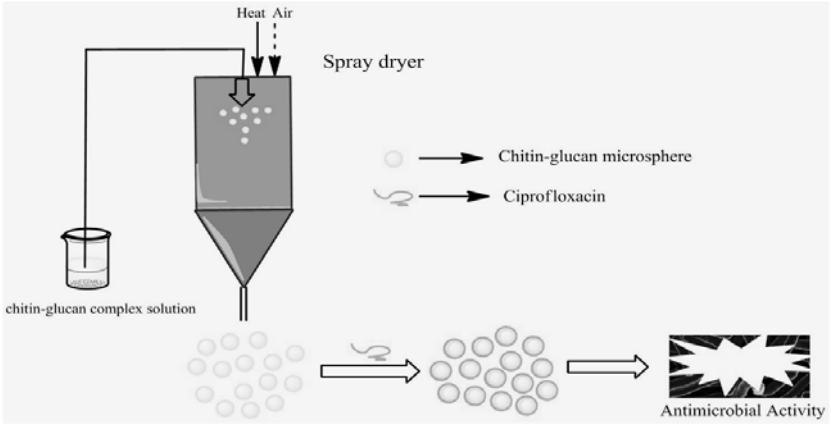
Formic acid was purchased from Merck, Nutrient broth, and Nutrient agar was purchased from Hi-media, *Bacillus subtilis,* and *Escherichia coli* colonies were received from IMTECH, Chandigarh. Ciprofloxacin was purchased from Sigma, India. Milli-Q was obtained from our department.

Preparation of Chitin-Glucan Microsphere

Chitin-glucan (ChG) microsphere was prepared by spray drying technique (LSD-48 MINI SPRAY DRYER JISL) with standard nozzle 0.7 mm and evaporation capacity 1.0 L/hr. Herein, 1 g of the chitin-glucan complex was dissolved in 100 mL of 5% formic acid and put overnight under stirrer. After a complete homogenous solution, the biocomposite solution was spray-dried at different parameters. Two experiments were performed at different temperatures. In the first experiment, inlet temperature was set at 145°C, Feed pump was set having range 15-20% then flow rate was measure around 4 ml/min, a jacketed temperature of 60-80°C and an outlet temperature of 40.6°C maintained by the aspirator. The pressure pump was used for the supplied of compressed air around 2- 5 bar, which was maintained at pressure regulator [10]. In the second experiment, all processes are the same only parameters are differently shown in Table 1. Finally, a very fine powder product was obtained in an airtight cyclone chamber. Then collecting the powder in the cyclone chamber and powder was stored in a container for further characterization.

TABLE 1. Parameter for the preparation of micro-spheres

Sample	Inlet Temp., °C	Outlet Temp., °C	Flow rate, mL/min	Mean particle size, µm	Reference
Chitin-glucan microsphere	160, 145	48, 40.6	2.5-4	1.5-3.4	Our Work
Chitosan microsphere	160	80-85	10	1.5-7.2	[18]



Scheme 1. Diagram of spray drying process of chitin-glucan solution

CHARACTERIZATION OF CHITIN-GLUCN MICROSPHERE

Crystallinity and amorphous nature of chitin-glucan microsphere was determined by X-ray diffraction (XRD) analysis having range 5 to 80° and the size of particle size was analyzed by (Microtrac Nanotrac Wave II) particle size analyzer instrument. Surface morphology was observed by scanning electron microscope (SEM) from IIT, Kanpur.

Evaluation of Antibacterial Activity Chitin-glucan and Ciprofloxacin Loaded Chitin-glucan Microsphere

Antibacterial activity of chitin-glucan and ciprofloxacin loaded chitin-glucan microsphere were evaluated by agar well diffusion method. [10,19,20] Bacterial strain were grown on nutrient broth then spread in solidified agar containing petri plates. After, spreading wells were created and samples were placed in holes and incubated for 12 h at 35°C Then finally zone of inhibition was analyzed and estimated.

RESULT AND DISCUSSION

X-ray Diffraction of Chitin-glucan Complex and Chitin-Glucan Microsphere

XRD diffraction of chitin-glucan complex and chitin-glucan microsphere were shown in Figure 1. From chitin-glucan complex we observed that peaks are crystalline in nature [2] and after spray drying crystallinity was reduced and surface become amorphous in nature.

Particle Size of Chitin-Glucan Microsphere

Particle size of chitin-glucan microsphere was analyzed by particle size analyser shown in Figure 2. Sample was dissolved in acidic solution and sonicate for few minutes after sonication solution was placed in particles size

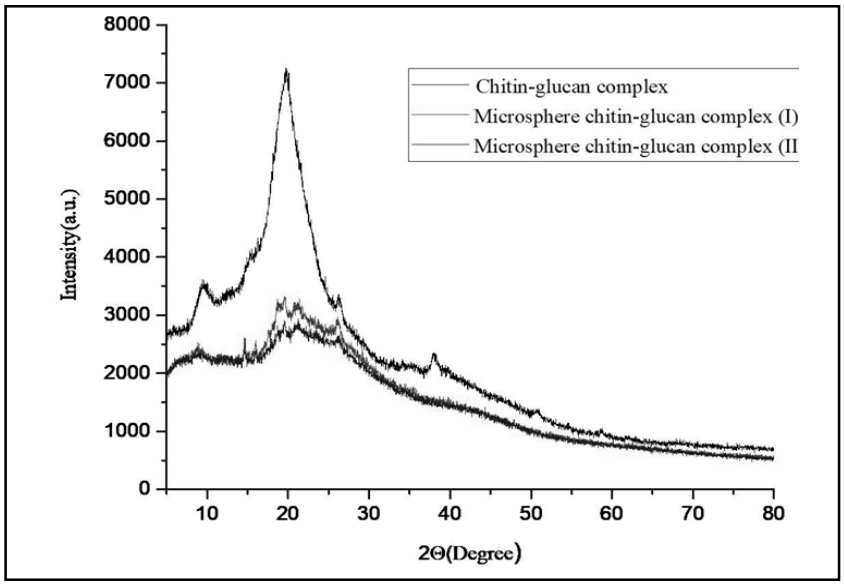
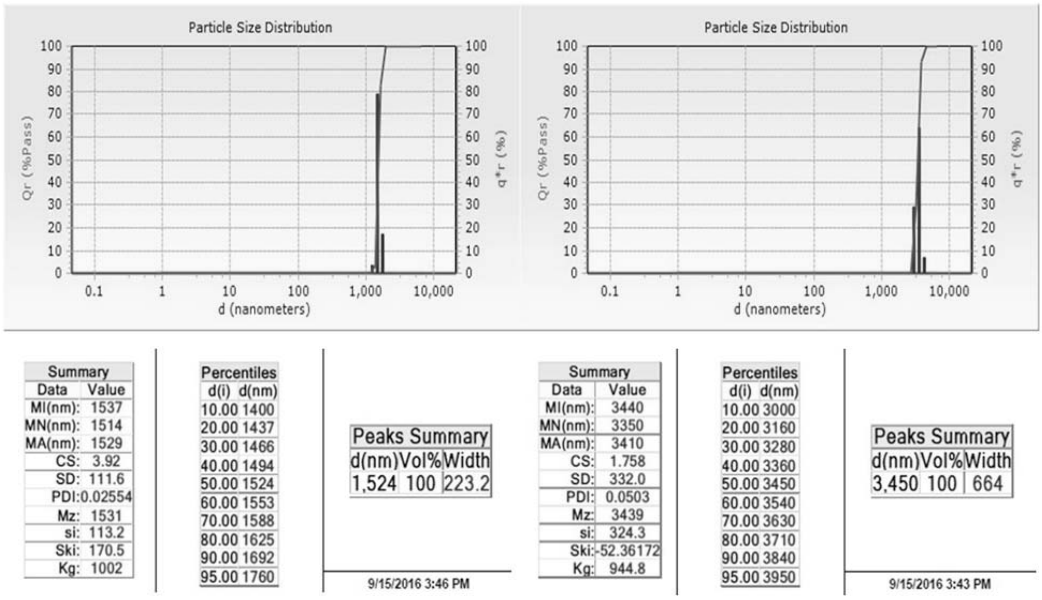


Figure 1. XRD pattern of chitin-glucan complex and chitin-glucan microsphere

analyser instrument. Then mean size was measured and range of chitin-glucan microsphere was observed around 1.5 µm in (I) and mean size around 3.4 µm in (II) condition.



Summary	
Data	Value
MI(nm):	1537
MN(nm):	1514
MA(nm):	1529
CS:	3.92
SD:	111.6
PDI:	0.02554
Mz:	1531
si:	113.2
Ski:	170.5
Kg:	1002

Percentiles	
d(i)	d(nm)
10.00	1400
20.00	1437
30.00	1466
40.00	1494
50.00	1524
60.00	1553
70.00	1588
80.00	1625
90.00	1692
95.00	1760

Peaks Summary		
d(nm)	Vol%	Width
1,524	100	223.2

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Summary	
Data	Value
MI(nm):	3440
MN(nm):	3350
MA(nm):	3410
CS:	1.758
SD:	332.0
PDI:	0.0503
Mz:	3439
si:	324.3
Ski:	-52.36172
Kg:	944.8

Percentiles	
d(i)	d(nm)
10.00	3000
20.00	3160
30.00	3280
40.00	3360
50.00	3450
60.00	3540
70.00	3630
80.00	3710
90.00	3840
95.00	3950

Peaks Summary		
d(nm)	Vol%	Width
3,450	100	664

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Figure 2. particle size analyser of chitin-glucan microsphere (I) & (II)

Morphology of Chitin-Glucan Microsphere

Scanning electron microscope of chitin-glucan microsphere was shown in Fig. 3. Chitin-glucan microsphere was shown nearly in spherical shape and surface become smooth [21].

Antibacterial Analysis

Antibacterial analysis of chitin-glucan and ciprofloxacin loaded chitin-glucan microsphere were shown in Fig. 4 (A) & 4 (B) having different concentration. These

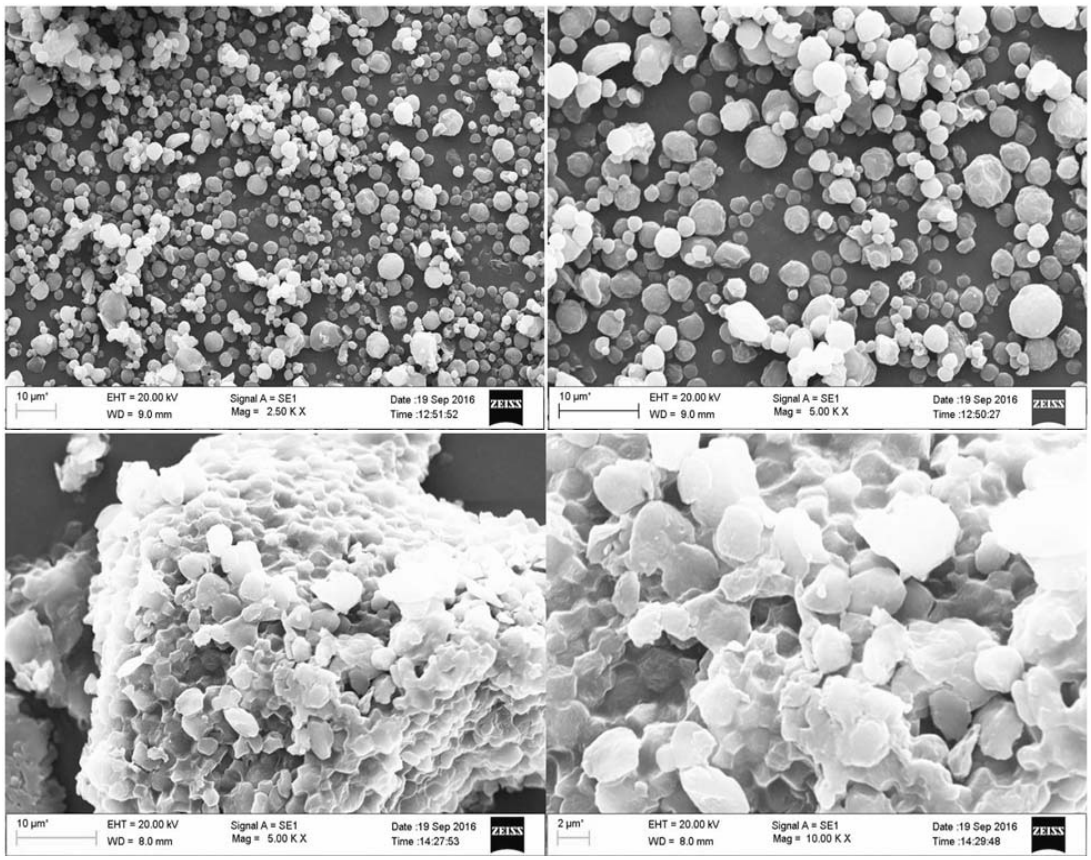

Figure 3. surface morphology of chitin-glucan microsphere (I) & (II)

TABLE 2. ZOI of chitin-glucan complex, chitin-glucan microsphere and ciprofloxacin loaded chitin-glucan microsphere

Test Cultures	ZOI in mm					
	Chitin-glucan complex			Ciprofloxacin loaded chitin-glucan		
Bacillus subtilis	5.0	6.7	8.1	17.0	20.0	21.5
Escherichia coli	6.5	8.0	8.8	15.0	21.0	25.0

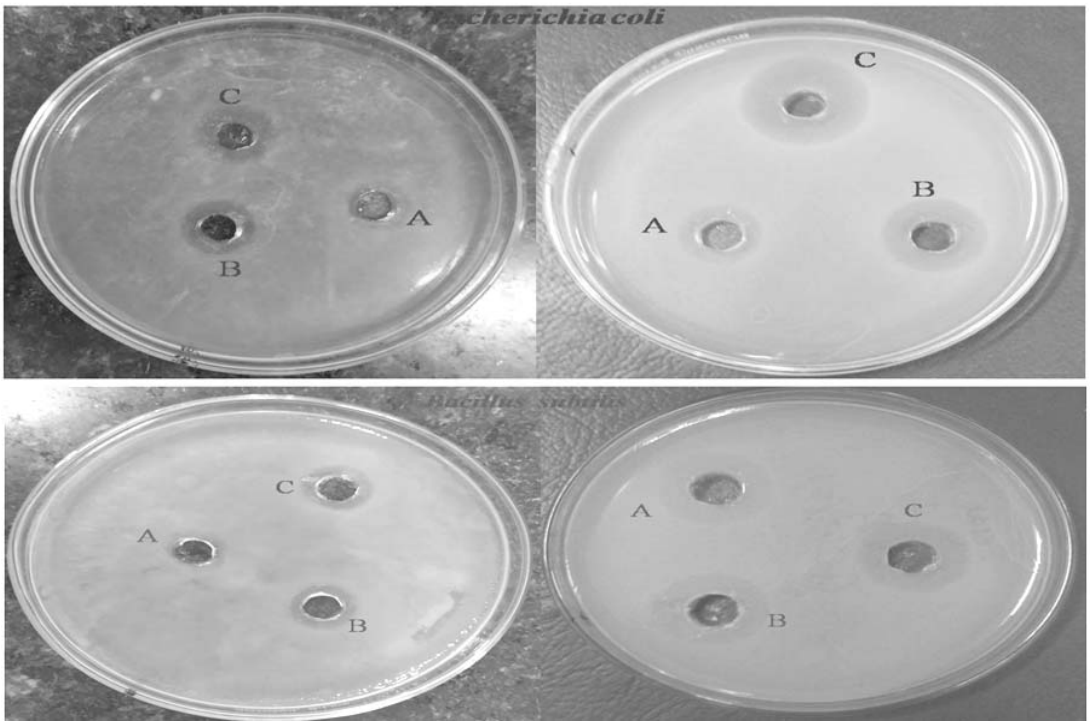

Figure 4. (A) & 4 (B) Antibacterial Activity (ZOI in mm) in Chitin-glucan and ciprofloxacin loaded chitin-glucan microsphere having different concentrations A, B, and C

images showed that ciprofloxacin loaded chitin-glucan microsphere completely inhibit in comparison to chitin-glucan. Therefore we can conclude that size of chitin-glucan decreases then antibacterial activity increases [10]. The value of ZOI in samples is given in summarized form of Table 2.

CONCLUSION

In the present work, we have developed the microsphere of the chitin-glucan complex with the help of spray drying technology. In this experiment, we have shown that the chitin-glucan microsphere (I) gives better characteristics property in comparison to the chitin-glucan microsphere (II). In this work, microsphere particles are used in antibacterial activity and also may be useful in many areas such as drug delivery and anticancer activity. Herein, we have loaded the ciprofloxacin in the chitin-glucan microsphere and increase the activity of the chitin-glucan microsphere. For spray drying techniques particles are spherical and the size of particles is around 1.5 to 3.5 µm. Herein, we have seen that in antibacterial activity ciprofloxacin loaded chitin-glucan microsphere completely inhibits the growth of bacteria in comparison to chitin-glucan. Also we have seen that chitin-glucan microsphere give better result in comparison to chitin-glucan complex.

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