Research Article

Inexpensive Apparatus for Fabricating Microspheres for 5-Fluorouracil Controlled Release Systems

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The aim of this study was to develop an inexpensive apparatus for fabricating microspheres, based on chitosan, for 5-fluorouracil (5-FU) controlled release. Chitosan microspheres were prepared by precipitation method and the effects of manufacturing parameters (injection and airflow rates) on size distribution microspheres were analyzed by optical and scanning electron microscopy. The results show that the manufacturing parameters, injection and airflow rates, determine the microsphere size distribution. By modulating these parameters, it was possible to produce chitosan microspheres as small as $437 \pm 44\,\mu$m and as large as $993 \pm 18\,\mu$m. Chitosan microspheres loaded with 5-FU were also produced using the experimental equipment. The obtained microspheres presented 5-FU controlled release, indicating that the microspheres can be used orally, since they are capable of crossing the stomach barrier and of continuing with the process of 5-FU release.

1. Introduction

Microspheres, particles that range in size from 50 nm to 2 mm in diameter, have application potential for drug-controlled release system and if they are bioadhesive they may be used for administration of drugs for localized action [1]. Due to a high surface to volume ratio, this kind of particle may present efficient absorption and enhanced bioavailability of the drugs [2]. By keeping the drugs in close proximity to their absorption window in the gastrointestinal mucosa, the bioadhesive microspheres improve the absorption and oral bioavailability of drugs, offering the possibilities of localized as well as systemic controlled release of drugs. This is due to the formation of noncovalent bonds such as hydrogen bonds and ionic interactions or physical entanglements between the mucus gel layer and mucoadhesive polymers [3, 4].

Over the last few years, the systems of controlled liberation of drugs based on biomaterials have attracted attention for the treatment of cancer. The biomaterials used on a large scale for the chemotherapeutics controlled release include natural polymers such as alginate, chitosan (CS), and cellulose derivatives. Among chemotherapeutic compounds used for cancer treatment, 5-fluorouracil (5-FU) is one of the most widely used antineoplastic drugs for the treatment of breast cancer [5], gastric cancer [6], pancreatic cancer [7], brain cancer [8], liver cancer [9], and colorectal cancer [10]. The 5-FU is a pyrimidine analog that inhibits the biosynthesis of deoxyribonucleotides for DNA replication by inhibition thymidylate synthase activity, leading to thymidine reduction, incorporation of deoxyuridine triphosphate into DNA, and cell death. A limitation of 5-FU use is the nonuniform oral absorption due to metabolism by dihydropyrimidine dehydrogenase present in the stomach [11]. Only intravenous preparations of 5-FU are available in market for clinical use. The 5-FU intravenous use presents disadvantages because it causes subcutaneous fat hypertrophy or atrophy and occasional allergies on the injection spot [12]. Furthermore, most of the side effects are due to exposure of drugs on undesired places. The systemic toxic effects, together with a short plasma half-life (10–20 min), make it necessary that these drugs have

to be handled by one local system capable of supplying a continues release [13].

The properties of the bioadhesive microspheres, that is, their surface characteristics, bioadhesion force, and release pattern of the drug, are influenced by the type of polymer used to prepare them, microsphere size/size distribution, morphology, and make-up. Varieties of synthetic or natural polymers have been employed as bioadhesive microspheres. Among them, biodegradable polymers have become increasingly important. A major advantage of these systems is that the degradation of polymeric materials could be achieved through the process of hydrolysis or enzyme-specific reaction. Among the enzymatically degradable polymers, chitosan, a cationic polysaccharide consisting of D-glucosamine and N-acetyl glucosamine, obtained from the chitin deacetylation process in an alkaline solution, has been extensively applied in drug delivery systems because it is biodegradable, biocompatible, nontoxic, nonimmunogenic, noncarcinogenic, antibacterial, and mucoadhesive. In addition, this polymer not only protects the drug molecules from degradation by proteolytic enzymes and prolongs the half-life time of drug, but also improves bioavailability of drug in vivo by controlling release rate of drug from the microspheres [1, 2, 12, 14–18].

Chitosan microspheres can be prepared by various methods such as cross-linking with anions, precipitation, complex coacervation, modified emulsification and ion tropic gelation [19], precipitation-chemical cross-linking, glutaraldehyde cross-linking, thermal cross-linking, emulsification/solvent evaporation, and spray drying [18, 20]. However, these processes present high cost. Thus, Dias et al. [21] and Prado et al. [22] performed studies on construction of an inexpensive apparatus for the production of chitosan microspheres. Nevertheless, this apparatus has limitations in controlling the chitosan solution injection flow, because the system provides a pressure gradient between the beginning and the end of the process. In order to overcome these limitations, the aim of this study was to develop an inexpensive apparatus for fabricating bioadhesive microspheres, based on chitosan and 5-FU, for oral controlled release systems, using an automated chitosan solution injection flow system. The improvement is the implementation of a low-cost electronic microcontroller (Arduino) and a mechanical system of linear injection (screw).

2. Materials and Methods

2.1. Materials. Commercial chitosan (deacetylation degree of 93%) was supplied by polymer (Fortaleza, Brazil) without prior purification. Sodium hydroxide, glacial acetic acid, and sodium acetate trihydrate were purchased from Vetec, Brazil. All aqueous solutions were prepared using distilled water and all reagents and solvents were of analytical grade and used as provided. The active duty of substance 5-fluorouracil, empirical formula $C_4H_3FN_2O_2$, molecular weight $130.1\,g\cdot mol^{-1}$, and purity $\geq 99\%$, was supplied by Sigma-Aldrich and phosphate buffer solution (PBS) acquired by Vetec Química Fina. The

microcontroller and man-machine interfaces were purchased from Shen Zhen Blue Sky Technology.

2.2. Preparation of Chitosan Microspheres. Chitosan microspheres were prepared by precipitation method employing experimental equipment developed in our lab (Figure 1). Briefly, chitosan solution (4% w/v) was prepared using an aqueous solution of acetic acid (5% v/v) containing 4% of sodium acetate. The solution was added dropwise, through a drip system constructed from polymeric pipe and a 0.45 mm diameter nozzle, into a gently stirred coagulation liquid (aqueous solution of sodium hydroxide, 8 v/v%). The effects of manufacturing parameters on the characteristics of the resulting microspheres were studied by setting the injection flow rate at $0.150\,mL\cdot min^{-1}$ (IR_1), $0.300\,mL\cdot min^{-1}$ (IR_2), and $0.600\,mL\cdot min^{-1}$ (IR_3) and the airflow rate at 2.5, 5.0, 7.5, and $10.0\,L\cdot min^{-1}$ (see Table 1 for details). The formed microspheres suspension was filtered and washed with distilled water until neutrality and then dried in an oven at 50°C for 24 h. Moreover, from time to time, the reactions of same sets of parameters were duplicated and the reproducibility was found to be excellent.

For the incorporation of 5-FU, microspheres produced with IR_1-2.5 and IR_1-10 were selected and named as CS/5-FU-2.5 and CS/5-FU-10, respectively. The dissolution of 5-FU (100 mg) was carried out in 10 mL of (4%) chitosan solution with addition of sodium acetate (400 mg) obtaining a 5-FU/chitosan/acetate ratio of 1 : 4 : 4. The dispersion was dripped into the coagulation liquid (aqueous solution of sodium hydroxide, 8 v/v%) and kept under low agitation. The formed microspheres suspension was filtered, with qualitative filter paper with open pores of $14\,\mu m$, and washed with distilled water until obtaining a pH of 7.5 and then dried in an oven at 50°C for 24 h.

2.3. Size Determination of Chitosan Microspheres. The size of the chitosan microspheres was determined from micrographs taken with a digital optical microscope (Q734ZT 059, DP Scientific Instruments). A small amount of dry microspheres was placed on a clean glass slide. The slide containing chitosan microspheres was mounted on the stage of the microscope and the obtained images of at least 10 particles were analyzed using Pixcavator 5 software. Average size (diameter, volume, surface area, and sphericity) and standard deviations of the microspheres on the micrographs were evaluated.

2.4. Microspheres Morphology. The surface topography of the microspheres was examined under a scanning electron microscope (Shimadzu SSY-550). A small amount of dry microspheres, at least 10 microspheres, was placed on aluminum stubs and made electrically conductive by coating with a thin layer of gold. A scanning electron photomicrograph was taken at the acceleration voltage of 30 KV and chamber pressure of 0.6 mm Hg.

2.5. Entrapment Efficiency of 5-FU into Chitosan Microspheres. The entrapment efficiency (EE) of 5-FU encapsulated into microspheres was determined by UV-Vis analysis (Perkin
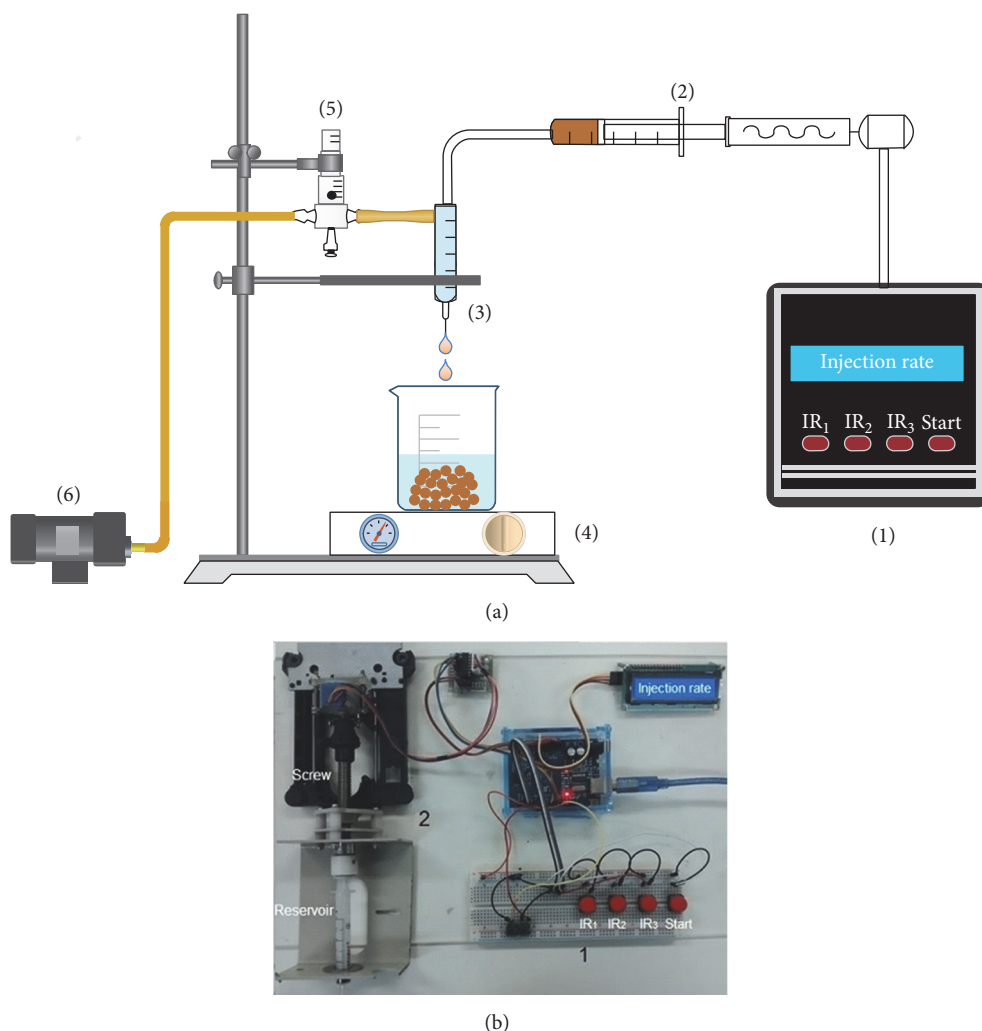
FIGURE 1: (a) Schematic diagram of the experimental equipment, used to prepare chitosan microspheres, developed in our lab: (1) electronic microcontroller, (2) injection zone, (3) dripper, (4) stirrer, (5) rotameter, and (6) pump. (b) The magnification, items 1 and 2, of the experimental equipment, used to prepare chitosan microspheres.

TABLE 1: Chitosan microspheres manufacturing parameters.

Airflow rate ($L\cdot min^{-1}$)	Injection flow rate 0.15 mL·min^{-1}	Injection flow rate 0.30 mL·min^{-1}	Injection flow rate 0.60 mL·min^{-1}
2.5	IR_1-2.5	IR_2-2.5	IR_3-2.5
5.0	IR_1-5.0	IR_2-5.0	IR_3-5.0
7.5	IR_1-7.5	IR_2-7.5	IR_3-7.5
10.0	IR_1-10.0	IR_2-10.0	IR_3-10.0

Elmer, Lambda 35). The nonassociated drug (free drug) was isolated from the microspheres by washing. The washing water was assayed for unbound drug content. Calculations were performed by using a calibration curve, and encapsulation efficiencies were determined as [23]

$$EE\ (\%) = \frac{total\ 5\text{-}FU - free\ 5\text{-}FU}{total\ 5\text{-}FU} * 100. \tag{1}$$

2.6. In Vitro 5-FU Release. 5-FU release from the chitosan microspheres was investigated in phosphate buffer solution (PBS) at pH 1.2. The amount of drug released was analyzed by UV spectrophotometry (Perkin Elmer, Lambda 35). In a typical release experiment, 500 mg of the 5-FU loaded microspheres was suspended in 50 mL of PBS. This suspension was placed in a shaking bath maintained at 37°C ± 0.5°C under continuous shaking conditions (100 rpm). Aliquots (5 mL) of the release media were withdrawn at predetermined time intervals (15 min until 48 h) and refilled immediately with the same volume of the fresh PBS. The concentration of 5-FU released from the drug delivery system was monitored by measuring the UV absorbance of the solution at 266 nm. The

cumulative drug release was calculated based on a standard calibration curve. All measurements were performed in triplicate and the results presented were the average of three runs.

2.7. Drug Release Kinetics. In order to understand the 5-FU release mechanism, the results obtained were adjusted with the kinetic model proposed by Korsmeyer-Peppas, which derived an expression for drug release kinetics [24]:

$$\frac{M_t}{M_\infty} = Kt^n + b, \tag{2}$$

where M_t/M_∞ is the fraction of drug released at time t, K is a rate constant, and n is a parameter characteristic of the release mechanism. Values of n between 0.5 and 1.0 indicate anomalous transport kinetics, and 0.5 indicates a Fickean diffusion controlled mechanism. Lower values of the exponent, $n < 0.5$, may be related to drug diffusion through an enlarged matrix or though water filled pores [25].

3. Results and Discussion

The automation of the injection flow rate with electronic microcontroller enabled monitoring, through Integrated Development Environment (IDE) and serial port, the regularity of the injection flow rate. According to Figure 2, the behavior of the injection flow rate was linear for the three studied injection flow rates: 0.15 mL·min^{-1} (IR$_1$), 0.30 mL·min^{-1} (IR$_2$), and 0.60 mL·min^{-1} (IR$_3$). This indicates that the pressure in the dripper injection nozzle was constant; that is, no pressure gradient between the beginning and the end of the process was detected. This allowed preparing chitosan microspheres with uniform size/size distribution using the apparatus developed in our lab, as can be proved by the diameter and sphericity values, determined from microspheres optical microscopy images analyses with the Pixcavator 5 software (Table 2).

Figure 3 shows the chitosan microspheres images obtained by optical microscopy. Both the injection and the airflow rates influenced the chitosan microspheres size/size distribution. The mean diameter of the dry microspheres ranged from 0.993 to 0.437 mm, depending on the combination injection/airflow rate employed to prepare the microspheres. The higher the injection and airflow rate, the smaller the microspheres size (Table 2).

The dependence of the microspheres diameter on the airflow rate and chitosan solution injection flow rate was modeled by a full second-order polynomial [26]. It was found according to expression:

$$Z = 1{,}566 - 1{,}039X - 0{,}187Y + 0{,}472X^2 + 0{,}008Y^2 + 0{,}090XY, \tag{3}$$

where Z is the microspheres diameter (mm), X is the airflow rate (L·min^{-1}), and Y is the chitosan solution injection flow rate (mL·min^{-1}) representing the experimental data adjustment error of 0.01206 and a correlation coefficient R^2 of 0.9412.

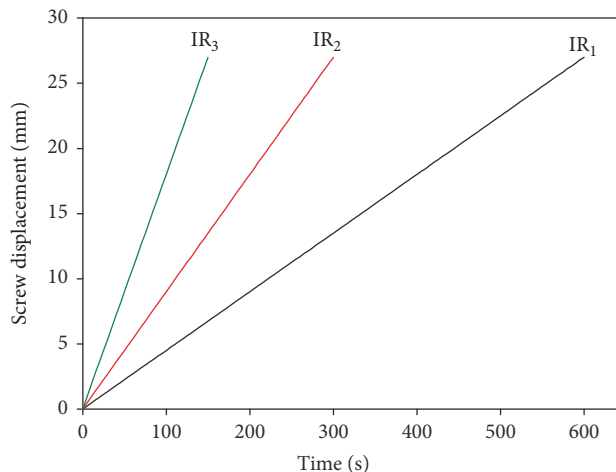Figure 2: Influence of screw displacement on injection flow rates regularity: 0.150 mL·min^{-1} (IR$_1$), 0.300 mL·min^{-1} (IR$_2$), and 0.600 mL·min^{-1} (IR$_3$).

Figure 4 shows the microsphere diameters as function the airflow rate for each injection flow rate (IR$_1$, IR$_2$ and IR$_3$). It is observed that a microsphere's diameter decreases when there is an increase in airflow rate. The air rate flowing parallel to the needle is the main responsible for the drop drag, preventing their growth. Thus, the bigger the airflow rate the larger the drag force, preventing the growth of droplet, resulting in a microsphere with smaller diameter [21].

The diameter of the microspheres prepared at IR$_1$ show a linear dependence on airflow rate, with a correlation coefficient of $R^2 = 0.994$. At IR$_2$ and IR$_3$ the dependence is clearly nonlinear, suggesting interference in the formation of the microspheres. The IR$_1$ injection flow rate is a better condition to obtain microspheres with chitosan solutions at 4% w/v. Thus, chitosan microspheres loaded with 5-fluorouracil (5-FU), a highly effective anticancer drug that has been widely used in the clinical treatment of numerous types of cancers [12], were prepared at IR$_1$ injection rate and at two airflow rates: 2.5 L·min^{-1} and 10.0 L·min^{-1}.

Figure 5 shows the scanning electron microscopy micrographs of chitosan and chitosan/5-fluorouracil (CS/5-FU) microspheres obtained at IR$_1$ injection rate and at two airflow rates: 2.5 L·min^{-1} and 10.0 L·min^{-1}. The CS and CS/5-FU particles prepared at 2.5 L·min^{-1} airflow rate presented regular shape and the smooth surface (Figure 5(a)). On the other hand, the microspheres obtained at higher airflow rate (10.0 L·min^{-1}) displayed shape and surface rather irregular (Figure 5(b)). The size of the CS/5-FU microspheres was similar to the CS microspheres. The diameters were 0.953 mm and 0.436 mm for CS/5-FU microspheres prepared at 2.5 L·min^{-1} and 10 L·min^{-1}, respectively, and were near to CS microspheres, 0.953 mm and 0.408 mm. These values are in agreement with those obtained by MO (Table 2).

Friction between air and the surface of the drop results in momentum transfer from the air to the drop, which decreases from the surface to the center of the drop. The resulting drag

TABLE 2: Size of chitosan microspheres.

| Manufacturing parameters	Diameter (mm)	Volume (mm^3)	Surface area (mm^2)	Sphericity
IR$_1$-2.5	0.993 ± 0.018	0.513 ± 0.028	3.568 ± 0.120	0.932 ± 0.011
IR$_1$-5.0	0.776 ± 0.047	0.247 ± 0.042	2.442 ± 0.093	0.880 ± 0.043
IR$_1$-7.5	0.615 ± 0.031	0.123 ± 0.018	1.558 ± 0.263	0.878 ± 0.036
IR$_1$-10.0	0.437 ± 0.044	0.045 ± 0.013	1.040 ± 0.497	0.795 ± 0.105
IR$_2$-2.5	0.987 ± 0.037	0.505 ± 0.058	3.483 ± 0.240	0.938 ± 0.004
IR$_2$-5.0	0.669 ± 0.010	0.157 ± 0.007	1.839 ± 0.303	0.881 ± 0.072
IR$_2$-7.5	0.494 ± 0.020	0.063 ± 0.007	0.908 ± 0.073	0.920 ± 0.033
IR$_2$-10.0	0.514 ± 0.098	0.077 ± 0.045	1.100 ± 0.298	0.870 ± 0.054
IR$_3$-2.5	0.832 ± 0.019	0.302 ± 0.021	2.480 ± 0.100	0.937 ± 0.005
IR$_3$-5.0	0.634 ± 0.051	0.135 ± 0.033	1.485 ± 0.322	0.928 ± 0.025
IR$_3$-7.5	0.560 ± 0.016	0.092 ± 0.008	1.254 ± 0.080	0.887 ± 0.043
IR$_3$-10.0	0.579 ± 0.015	0.102 ± 0.008	1.329 ± 0.103	0.892 ± 0.023
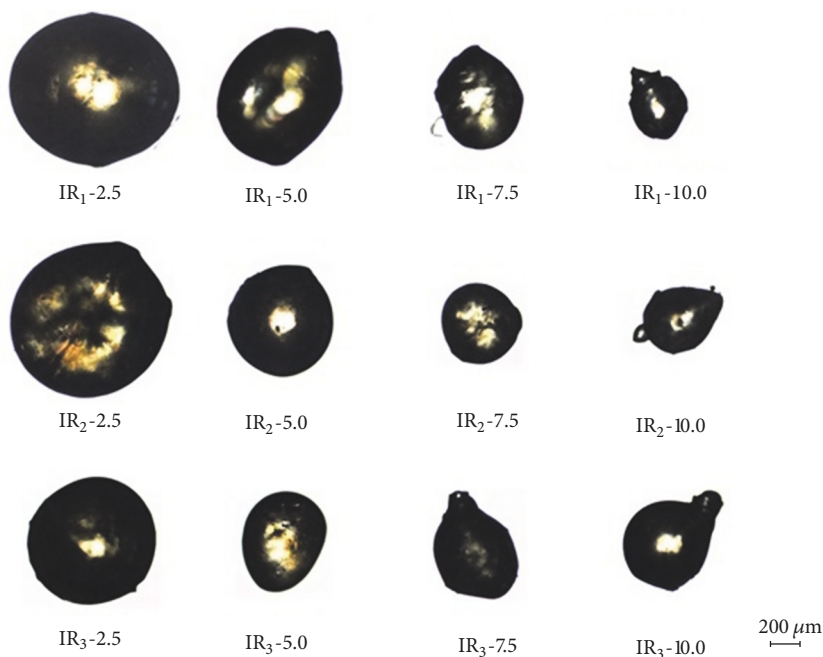
FIGURE 3: Chitosan microspheres optical microscopy images prepared at three injection flow rates: 0.150 mL·min^{-1} (IR$_1$), 0.300 mL·min^{-1} (IR$_2$), and 0.600 mL·min^{-1} (IR$_3$) and four airflow rates (2.5, 5.0, 7.5, and 10.0 L·min^{-1}).

force (F_y) is proportional to the drop surface area (A) and to the airflow velocity gradient (dV_y/dx), as follows:

$$\frac{\left|F_y\right|}{A} = k \cdot \frac{dV_y}{dx},\tag{4}$$

where k is a constant associated with the properties of the injected chitosan solution. Equation (4) indicates that the gradient of airflow velocity is inversely proportional to the drop surface area, which in turn is proportional to the square diameter of the drop, as shown in Figures 4 and 5. Drop formation at the exit of the injection needle follows a similar pattern.

Microspheres produced with IR$_2$ and IR$_3$ are smaller than those obtained with IR$_1$ up to an airflow rate of 7.5 L·min^{-1}, when the flow regime at the injection point becomes turbulent.

The entrapment efficiency (EE) of 5-FU encapsulated into the CS/5-FU-2.5 and CS/5-FU-10 microspheres was found to be 37.29 and 29.37% (Table 3), respectively. Previous works show that the entrapment efficiency of 5-FU is between 28–66% [27] and 29–69% [28]. In this study, the results obtained are in good agreement with these works. The microspheres size affected the 5-FU entrapment efficiency. This observation was based on quantification of the nonassociated 5-FU (free 5-FU). The amount of 5-FU into CS/5-FU-2.5 and

TABLE 3: 5-FU encapsulation efficiency data.

Sample	Equivalent diameter (mm)	Surface area (mm$^2 \cdot$g^{-1})	Total 5-FU (mg)	Free 5-FU (mg)	EE (%)
CS/5-FU-2.5	0.836 ± 0.042	1428.0 ± 227.9	69.44	43.54	37.29
CS/5-FU-10	0.451 ± 0.014	2138.9 ± 302.5	65.88	46.53	29.37

FIGURE 4: Diameter of the microspheres prepared at different injection flow rates.

TABLE 4: Parameters of regression of Korsmeyer on the liberation 5-FU in PBS of pH 1.2.

Sample	K	n	b	R^2
CS/5-FU-2.5	7.56	0.01	−7.79	0.9571
CS/5-FU-10	5.09	0.01	−4.97	0.8256

other hand, 5-FU was absorbed onto the external surface of the chitosan microspheres. These results show that the release rate of 5-FU from the microspheres in PBS (pH 1.2) can be controlled by the manufacturing parameter. In addition, the difference between the release profiles may be related to the microspheres surface area, resulting in a higher 5-FU release for the CS/5-FU-10 formulation. The data was in accordance with the results of Akbuga and Bergisadi [30] and Zhang et al. [31].

The exponent values n of the equation of Korsmeyer for the release process of 5-FU in pH of 1.2 are presented on Table 4.

The values of n presented on Table 4 indicate that the 5-FU release from the CS/5-FU-2.5 and CS/5-FU-10 formulations occurs predominantly due to the diffusion mechanism [32].

4. Conclusions

In the present study was developed an inexpensive apparatus for manufacturing microspheres based on chitosan for drug-controlled release, using an automated chitosan solution injection flow system. The results show that the manufacturing parameters, injection and airflow rates, determine the microsphere particle-size distribution. By modulating these parameters, it was possible to produce chitosan microspheres as small as 437 ± 44 μm and as large as 993 ± 18 μm. The chitosan microspheres obtained by this method were homogeneous in size with small deviation, especially when the smaller injection and airflow rates were used. Moreover, these sizes were reproducible between experiments. It was possible to produce chitosan microspheres loaded with 5-fluorouracil (5-FU), a highly effective anticancer drug that has been widely used in the clinical treatment of numerous types of cancers, by the experimental equipment developed in our lab. The microspheres obtained presented controlled release properties suggesting that they may be used in the 5-FU oral administration, since it is possible for them to cross the stomach barrier and continue the release process.

It should be noted that in the present work drop formation was studied under controlled conditions. Real world systems may involve the presence of contaminants, as well
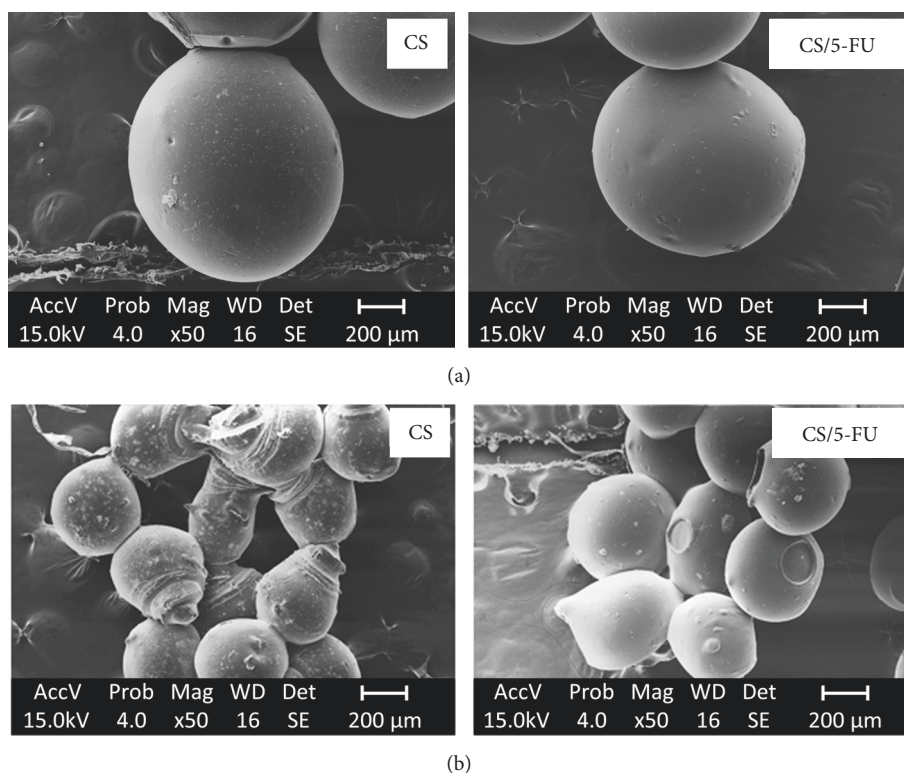
CS/5-FU-10 sample washing water was 43.54 and 46.53 mg (Table 3), respectively, indicating that the higher the surface area of the microspheres, the higher the drug lost during the washing process.

In order to investigate the effect of microspheres manufacturing parameter (airflow rate) on the 5-FU release, the *in vitro* release of CS/5-FU-2.5 and CS/5-FU-10 formulations was carried out in a buffer solution of pH = 1.2 by UV spectrophotometry. Figure 6 demonstrates 5-FU release profiles up to 48 h of incubation period. As shown in this figure, chitosan microspheres presented an initial burst release of 5-FU in a period of 390 min, which was in the range of 88% for CS/5-FU-2.5 and 98% for CS/5-FU-10. This initial rapid release, characterized as "burst effect," is due to the fact that part of the drug was entrapped close to or at the surface of the chitosan microspheres, which could be easily released by diffusion [12, 29]. After the initial burst effect, a slower, sustained, and controlled release occurred throughout the incubation period, which may involve the diffusion of the 5-FU entrapped within the inner part of chitosan microspheres, through the pore network. The second and slower release was in the range of 12% for CS/5-FU-2.5 and 2% for CS/5-FU-10. Release profiles are consistent with the encapsulation of 5-FU among the positively charged hydrophilic chains. On the

(a)

(b)

FIGURE 5: Microspheres prepared at 0.15 mL·min^{-1} injection flow rate (IR$_1$) and two airflow rates: (a) 2.5 L·min^{-1} and (b) 10.0 L·min^{-1}.
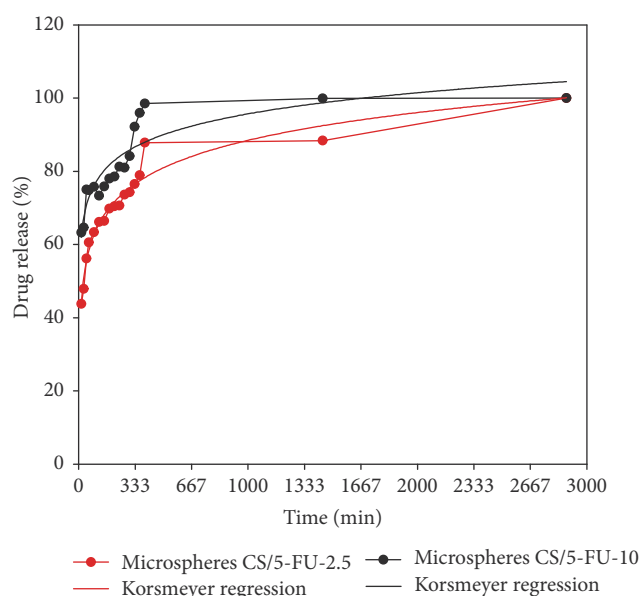

FIGURE 6: Liberation profile of 5-FU from microspheres in PBS of pH 1.2.

as pH, temperature, viscosity, and surface tension variations that could affect the results. Future research based on the apparatus developed in this contribution may address these issues.

Conflicts of Interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest.

Acknowledgments

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