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PREPARATION AND CHARACTERIZATION OF pH/TEMPERATURE SENSITIVE MICROCAPSULES BY INVERSE EMULSION POLYMERIZATION

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Keywords:

pH/temperature sensitive microcapsules, inverse emulsion polymerization, sodium alginate, N-isopropyl acrylamide, drug release properties

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ABSTRACT: The pH/temperature sensitive microcapsules were prepared by inverse emulsion polymerization, in which sodium alginate (SA) and N-isopropyl acrylamide (NIPAm) were used as the wall material and pentoxifylline as a model drug. The effects of the emulsifier content on the particle size distribution and drug release property of microcapsules were discussed in detail. The structure, morphology, particle size and its distribution of microcapsules were characterized by Fourier transform infrared spectrophotometry (FTIR), scanning electron microscopy (SEM), transmission electron microscope (TEM), Malvern particle size analysis and UV-vis spectrophotometer. The release behavious of pentoxifylline in different phosphate buffered saline (PBS) pH were investigated systematically. The results showed that the resultant microcapsules had narrower particle size distribution, smoother surface and higher drug release amount with the percentage weight of emulsifier being 2.0%. The TEM photograph exhibited that the microcapsules had core and shell structures. The drug-release behavior of pentoxifylline from microcapsules was evaluated as a function of pH/temperature. The results indicated that these drug-loaded microcapsules have shown sensitivity to pH value and temperature.

INTRODUCTION: In the recent years, intelligent polymers have attracted increasing interests due to their potential and biomedical applications such as drug delivery system [1], bio-separation [2], cell culture [3] and enzyme technology [4]. The intelligent microcapsule can respond to external stimuli changes such as temperature [5], pH value [6], ionic strength [7], light [8] and electric fields [9], etc. Among the intelligent microcapsules, pH/ temperature sensitive microcapsules have been widely investigated in the biomedical fields due to the easy controlling and applications both *in vitro* and *in vivo* conditions [10, 11].

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Among the temperature-sensitive polymers, poly (N-isopropylacrylamide) (PNIPAm) is extensively studied owing to the biocompatible property and its lower crystal solution temperature (LCST) around 32°C [12-14], which is close to human body temperature. Therefore, PNIPAm has been widely investigated in order to exploit its biomedical applications.

The pH sensitivity of polymer is usually originated from the introduction of carboxyl (-COOH) or amine (-NH2) groups into macromolecular chains [15].

As a result, acrylic acid (AA), methacrylic acid, vinyl pyridine, aminoethyl acrylylate, and so on are often selected as the comonomers or modifier in the preparation. Among the pH-sensitive polymers, sodium alginate (SA) is a linear polymer comprising 1, 4-linked-β-d-mannuronic acid (M) and α-L-guluronic acid (G) units, combined in blocks of M-M, G-G and M-G [16].

It has been widely used in the food, pharmaceutical, and cosmetic industries due to its biocompatibility, biodegradability, non-toxicity, non-immunogenicity, and acceptance by human body. SA is hydrophilic, absorbing water quickly. Its -COOH and -OH groups participate in hydrogen bonding and van der waals force [17-19].

Among the several approaches used in order to prepare microcapsules, the inverse emulsion polymerization is one of the most feasible methods. In the inverse emulsion polymerization, the non-polar liquid (such as hydrocarbon solvent) is used as the continuous phase as well as the water-soluble monomer used as dispersed phase. The water-soluble monomer is successfully dispersed in the continuous phase using a water-in-oil emulsifier, leading to the formation of water in oil (W/O) emulsion. Inverse emulsion polymerization confers the benefits of emulsion polymerization kinetics such as the rapid polymerization rates combined with high polymer molecular weights on water-soluble polymers [20].

In this study, the pH/temperature sensitive microcapsules with sodium alginate (SA) and N-isopropyl acrylamide (NIPAm) as wall-forming material were prepared by inverse emulsion polymerization, in which pentoxifylline was used as a model drug and span20 was used as the emulsifier. The effects of the emulsifier content on the particle size distribution and drug release property of microcapsules were discussed in detail. The release behavious of pentoxifylline in different phosphate buffered saline (PBS) pH were investigated systematically. The aim of this study is to gain a better understanding of the sodium alginate grafted N-isopropylacrylamide to preparation microcapsules, which layed foundation for further research on pH/temperature sensitive microcapsules.

2. MATERIALS AND METHODS:

2.1. Materials: Sodium alginate (SA) purchased from Tianjin Guangfu Fine Chemical Reagent Factory and N-isopropyl acrylamide (NIPAm) purchased from Dongjing Huacheng Co. were used as wall-forming materials. Pentoxifylline purchased from Dongjing Huacheng Co. was used as a core material. N, N-methylenebis acrylamide (MBA) obtained from Tianjin Kermel Chemical Reagent Co. was used as a crosslinker. Ammonium cerium nitrate (CAN) purchased from Tianjin Kermel Chemical Reagent Co. was used as an initiator. White oil obtained from Tianjin Jinfeng Chemical Reagent Co. was used as continuous phase. Span20 obtained from Jiangsu Haian Petroleum chemical Reagent Factory was used as an emulsifier. All the purchased reagents were in analytical grade.

2.2. Preparation of microcapsules: 20 ml 1% solution of sodium alginate with 1.8 g N-isopropyl acrylamide, 0.12 g N, N-methylenebis acrylamide and 0.5 g pentoxifylline was prepared by an adequate mixture. The aqueous solution was poured into oily solution with 100 ml white oil and 3 ml Span 20. The mixture was then vigorously agitated at 8000 r/min for 8 min with a homogenizer (BME100L, Shanghai Weiyu Co. China) to obtain w/o emulsion. 30 mL of 0.2% ammonium cerium nitrate aqueous solution was then slowly added into the w/o emulsion. The procedure was carried out at 40 °C with agitation at 100 r/min using a magnetic stirrer (Tokyo Rikakikai Co., Ltd.). Polymerization and microencapsulation were attained with continuous agitation at 100r/min for 5 h. The obtained microcapsule slurry was first washed with ethyl acetate and recentrifuged three times at 9000 r/min for 12 min, and then washed with distilled water to remove free N-isopropyl acrylamide and pentoxifylline on their surfaces, then freezed drying for at least 72 h.

2.3. Characterizations: The IR spectra were recorded with a Fourier transform infrared spectrophotometer (Vector22, Bruker Co., Germany). The sample were ground with dried KBr powder, and compressed into a plate. The KBr plate was scanned by a FTIR spectrophotometer. The morphology of the microcapsules was observed by scanning electron microscopy (SEM, JSM-5400, JEOL, Japan). Microcapsules were sprinkled onto a double-sided tape, sputter-coated with gold and examined in the microscope using the accelerated voltage of 10kV. The internal structure was observed by transmission electron microscopy (TEM, Tecnai G^2 F20 S-TWIN, FEI). The microcapsules were dispersed in the water, then remove the well dispersed microcapsules out of the water with a copper mesh which was clipped by a pair of pliers and dry at 50 °C.

The average diameter and size distribution were measured by particle size analyzer (Zetasizer 3000HS, Malvern Instruments, UK). Before measuration, the samples were diluted with hexane at 1:10 volume ratio. The amount of released pentoxifylline was measured by monitoring the optical absorbance at 274 nm using a UV-vis spectrophotometer (UV-2550, Shimadzu Co., Japan).

2.4. *In-vitro* drug release property: In a flask, the pentoxifylline-containing microcapsules (0.0500 g) were immersed into 100 mL PBS (pH7.6 or 1.2) simulated to the gastric fluid and neutral intestine. The release system was thermostated in a shaking bath at a designated temperature. At scheduled time intervals, 3 mL sample solution was withdrawn from the release medium. The pentoxifylline released from microcapsules was measured by the absorption at 274 nm using a UV-vis spectrophotometer. Then, the measured sample solution was returned to the flask to maintain the unchanged volume. Finally, the amount of the released pentoxifylline from microcapsules was calculated according to the standard calibration curve constructed from a series of pentoxifylline solution with standard concentration [21, 22].

3. RESULTS AND DISCUSSION:

3.1. Effects of the emulsifier content on the particle size distribution: The particle size of each microcapsule depends on the size of the dispersed droplet, which is determined by the used emulsifiers and the emulsifying conditions [23]. To study the effects of the emulsifier content on the particle size and particle size distribution of microcapsules, the emulsifier contents vary from 1.0% to 2.5%. In the experiment, the other reaction conditions do not change and only the emulsifier content increase in the reaction system [24]. Figure 1 illustrates the particle size distribution of the microcapsules at different amounts of emulsifier. The average particle size of the microcapsules for 1.0%, 1.5%, 2.0% and 2.5% of emulsifier content are 184.0, 152.9, 80.0 and 252.8 nm, respectively. As the emulsifier contents increase from 1.0% to 2.0%, the average particle size of resultant microcapsule becomes smaller and size distribution becomes narrower. When the emulsifier content is too low, it is easy to occur aggregation, resulting in a bigger size and broader particle size distrubution. When the emulsifier content was 2.0%, the average particle size of resultant microcapsule is the smallest and size distribution is the narrowest. As the emulsifier content continues to increase, the average particle size of resultant microcapsule becomes bigger and size distribution becomes broader. The reason may be that excessive emulsifier molecules contain excessive monomers [25]. Therefore, excessive monomers can be reacted rapidly on the surface of the cores to cause coalescence among the particles.

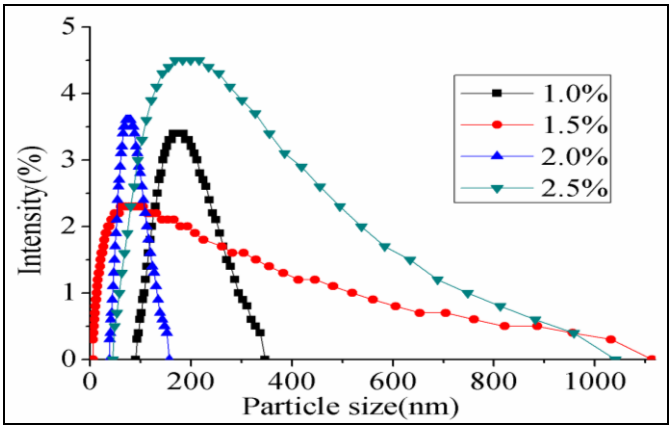

FIG. 1: PARTICLE SIZE DISTRIIBUTION OF MICROCAPSULES PREPARED AT DIFFERENT CONCENTRATIONS OF EMULSIFIER

3.2. Morphology of microcapsules: The SEM photograph of the emulsifier content of 2.0% was shown in **Fig. 2**. As showed in **Fig. 2**, the microcapsules revealed smooth surface and spherical shape. **Fig. 3** showed the TEM photograph of microcapsules. As shown in **Fig. 3**, it exhibited that the microcapsules have core and shell structures. Further, it was identified the formation of pentoxifylline inside the microcapsules.

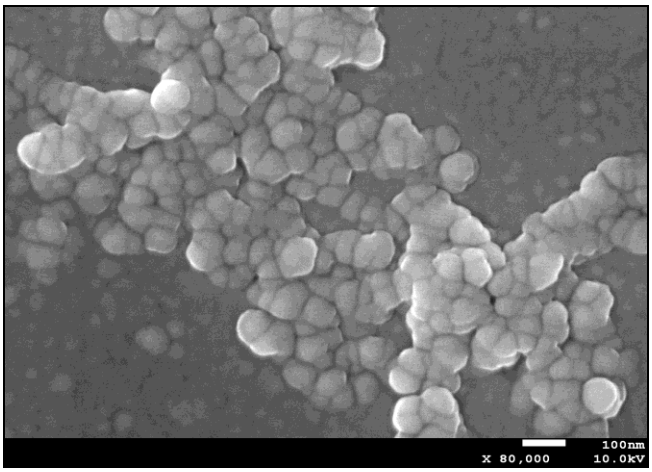

FIG. 2: SEM PHOTOGRAPH OF MICROCAPSULES

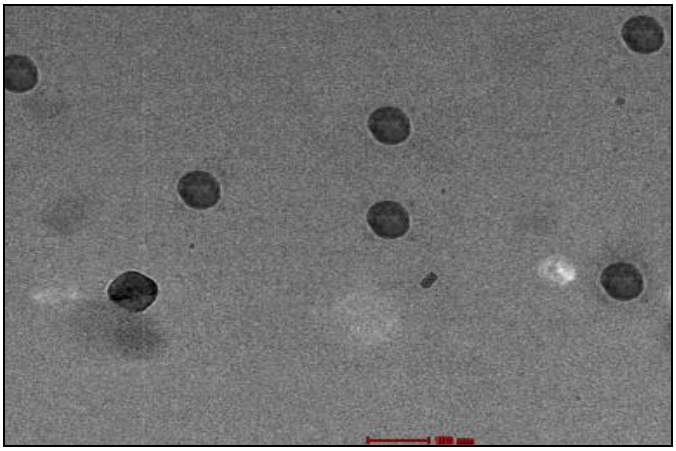

FIG. 3: TEM PHOTOGRAPH OF MICROCAPSULES

3.3. Structure of microcapsules: FTIR spectroscopy results for the samples are shown in **Fig. 4.** As we can see from the spectrum of Figure 4a, The characteristic peaks of pentoxifylline are as follows: C=O stretching vibration of amide bond (1700 cm^{-1}), C=O stretching vibration of ketone (1719 cm^{-1}), N-H bending vibration of amide (1548 cm^{-1}), C-N out of plane wagging of amide (752 cm^{-1}) and C-N stretching of amide (near 1410 cm^{-1}). As shown in Figure 4b, adsorption peaks at 1541cm^{-1} was assigned to the N-H stretching, C=O stretching at 1458cm^{-1} and 1648cm^{-1} were also observed. The former is $-COO^{-}$ symmetric stretching vibration peak and the latter is $-COO^{-}$ antisymmetry stretching vibration peak. The two peaks are the characteristic absorption peak of sodium alginate, which demonstrates SA and NIPAm are grafting together. In Figure 4c, C-N out of plane wagging of amide at 752 cm-1 was the characteristic peak of pentoxifylline, proving that the pentoxifylline was encapsuled in the microcapsules [26].

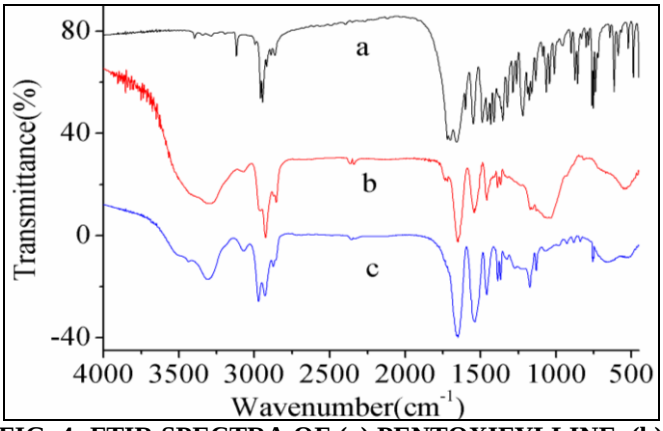

FIG. 4: FTIR SPECTRA OF (a) PENTOXIFYLLINE, (b) SA-NIPAm AND (c) PENTOXIFYLLINE-CONTAINING MICROCAPSULES.

3.4. Effect of pH on the drug release behaviour of microcapsules: As shown in Figure 5, in vitro release, the amount of pentoxifylline release is much higher in alkaline solution than that in acidic solution. This behavior may be attributed to the following fact: the carboxyl groups from the microcapsule wall turned into $–COO^{-}$ form in the pH 7.6 PBS.

The higher repulsion among -COO- groups induced to larger porous size for the drug release, resulting in a higher amountof pentoxifylline release. In pH 1.2 PBS, the ionization of carboxyl groups is low, so the stronger H-bonds between carboxyl groups led to the lower drug release [27]. The results showed that these sensitive drug-loaded microcapsules can bypass the acidity of gastric fluid without liberating the substantial drug and release a mass of drug in the small intestine.

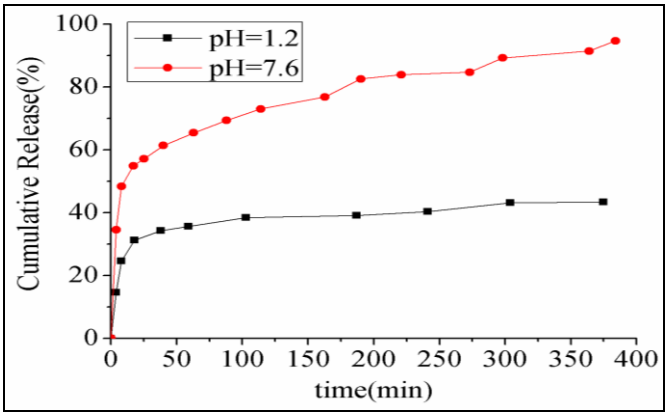

FIG. 5: RELEASE PROPERTIES OF MICROCAPSULES IN DIFFERENT PBS pH. RELEASE CONDITIONS: TEMPERATURE: 25.0 °C.

3.5. Effect of temperature on the drug release behaviour of microcapsules: Fig. 6 shows the vitro release properties of pentoxifylline from microcapsules in pH 7.6 at different tenperature. As shown in **Fig. 6**, the amount of pentoxifylline release is higher at 25°C solution than that at 37°C in the same PBS pH. This behavior may be attributed to the following fact: SA/PNIPAm chains containing hydrophilic amide groups ($-CONH^{-}$), the stronger H-bonds between $-CONH^{-}$ and water are the main reason to the lower drug release above the polymer phase transition temperature [28]. When the microcapsules are heated to a temperature above 32°C, the H-bonds between the microcapsule shell material are break, leading to the contraction of wall-forming material.

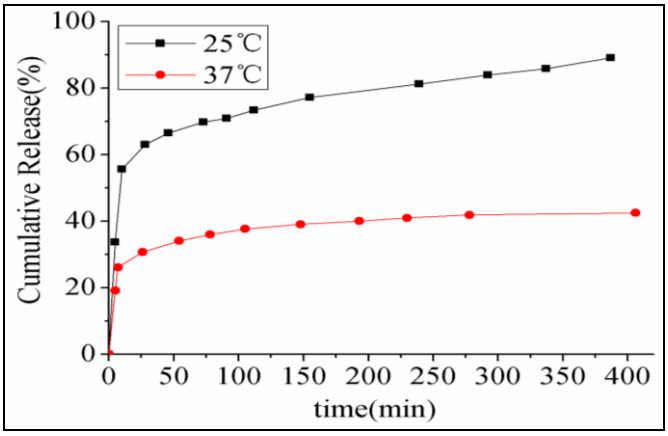

FIG. 6: RELEASE PROPERTIES OF MICROCAPSULES AT DIFFERENT TEMPERATURE. RELEASE CONDITIONS: pH 7.6.

3.6. Effect of emulsifier content on the drug release behaviour of microcapsules: The effect of emulsifier content on pentoxifylline release was conducted in PBS of pH 7.6 at 37 °C. As shown in **Fig. 6**, the pentoxifylline release was the highest at the emulsifier content of 2.0%, while this at the emulsifier content of 1.0%, 2.0% and 2.5%, was lower. The reason should be that the average particle size of the microcapsules at the emulsifier content being 2.0% is the smallest, while those from the microcapsules at the emulsifier content being 1.0%, 1.5% and 2.0 % are larger, and the smallest particle size microcapsules would have the largest total specific surface area, therefore causing the highest release at the same temperature and pH [29].

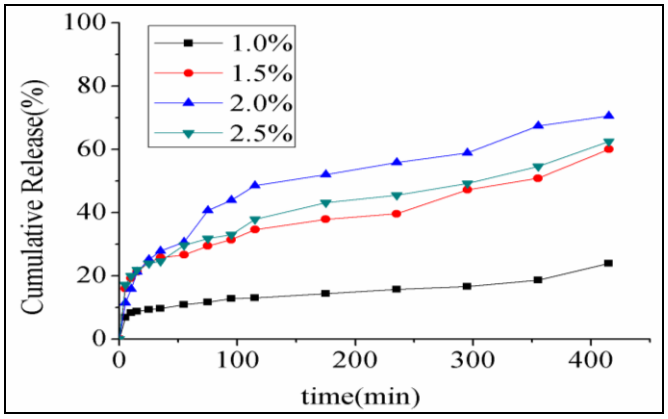

FIG. 7: RELEASE PROPERTIES OF MICROCAPSULES AT DIFFERENT EMULSIFIER CONTENT. RELEASE CONDITIONS: pH 7.6 AND TEMPERATURE: 37.0 °C.

CONCLUSION: The pH/temperature-sensitive microcapsules were synthesized by inverse emulsion polymerization, in which alginate and N-isopropyl acrylamide were used as wall-forming materials and pentoxifylline as a model drug. The FTIR analysis of pentoxifylline containing microcapsules demonstrated that pentoxifylline was successfully encapsulated in the microcapsule. The resultant microcapsules had narrower particle size distribution, smoother surface and higher amount of pentoxifylline release with the emulsifier being 2.0%. The TEM photograph exhibited that the microcapsules had core and shell structures. The drug-release behavior of pentoxifylline from microcapsules was evaluated as a function of pH and temperature.

The results indicated that these pH/temperature-sensitive drug-loaded microcapsules can bypass the acidity of gastric fluid without liberating the substantial drug, and release a mass of drug in the small intestine. As a result, these drug-loaded microcapsules showed both pH sensitivity and temperature sensitivity.

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CONFLICT OF INTEREST: The author declares that there is no conflict of interest.

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