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PREPARATION AND DRUG-LOADING PERFORMANCE OF TARTARY BUCKWHEAT STARCH MICROSPHERE

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ABSTRACT

Neutral starch microsphere from tartary buckwheat starch was firstly synthesized by inverse microemulsion technology with epichlorohydrin as a crosslinker and pan-60 as emulsifier and soluble tartary buckwheat starch as starting material. The absorption characteristics and mechanisms of starch microsphere for methylene, carminum and lemon yellow were also explored. The results showed that starch microsphere was around ball shape with rough surface. The size distribution of it was in the range of 20-75 μm with an average diameter of 32 μm. Three kinds of dyes were rapidly adsorbed by starch microsphere 60 minutes before and the adsorption processes reached equilibrium in about 100 min. The adsorption equilibrium data of three dyes correlated well with the Langmuir isotherm model and fitted well pseudo-second-order kinetic model.

Keywords: Buckwheat Starch, Starch Microsphere, Inverse Suspension Polymerization

INTRODUCTION

Starch microspheres are artificial starch derivatives from natural starches by various methods such as physical, chemical, enzymatic hydrolysis, and inverse microemulsion technologies. They have been the most interesting investigating materials as drug carriers and adsorbents for their biocompatibility, biodegradability, nontoxicity, stability on storage, cost effectiveness and simple product in technology **[1]**, and have been used successfully in nasal drug delivery system, artery embolization studies, radiotherapy, immuno-assay, etc **[2]**. The source of starch and preparation methods of starch microsphere affect the size distribution, irregular appearance, suitable swelling, specific surface area of starch microsphere, which further have an effect on distribution in organism, drug-loading performance, half life periods of biodegradation of them **[3]**.

Thus, it is very important to prepare starch microsphere with suitable methods and to study the adsorption properties and thermodynamics towards metal ion, followed by the study of adsorption mechanism towards metal ions as an important work. The materials of preparating starch microspheres were mainly rice, tomato, corn and cassava. Tartary buckwheat (Fagopy-rum tataricum)), cultivated in cool temperate area from 2500 m-3500 m in Southwest China for long history, contain many nutrients such as protein, vitamin, starch, essential amino acids, phytosterols, minerals (zinc, magnesium, calcium, etc) and functional ingredients such as (flavonoids, quercetin, fagopyrins and rutin, ect., which are not found in other cereal crops) **[4]**, and much attention has recently been paid to separating protein or rutin for the use of functional food or medicine. Many corresponding products such as functional protein with immunity-enhancing and fatigue-resisting and blood lipid-lowering activity have been sell well all over the country except alcoholic beverages, pasta, blended bread and noodles. The starch remains, generated during the protein and rutin processing, were discarded and formed the new waste of resource and pollution of exterior circumstance. The tartary buckwheat starch has a similar physical properties including structure and particle size distribution, swelling power and solubility, water-binding capacity and gelatinization temperature as starches obtained from other cereals such as potato, maize, and cassava **[5, 6]**. However, limited information about profoundly process and application of starch from tartary buckwheat was reported.

Therefore, the aim of this study was to investigate the absorption characteristics and mechanisms of tartary buckwheat starch microsphere for alkaline drug, acid drug and neutral drug. For this purpose, tartary buckwheat starch microsphere was prepared by emulsification-crosslinking technology, using epichlorohydrin as crosslinking agent, and drug-loading performance and adsorption kinetics of it was also explored using methylene (MB, alkaline drug), carminum (CA, neutral drug) and lemon yellow (LY, acid drug) as model drug.

MATERIALS AND METHOD

Materials

Tartary buckwheat was purchased from agricultural trade market. Epichlorohydrin (Tianjin Chemical Co.), Span60 (GuoYao Chemical Co.), methylene, carminum and lemon yellow, all reaction reagents are analytical and they have been used without further purification

Starch Separation From Tartary Buckwheat

Tartary buckwheat starch was separated from the flour as before reported methods with some modifications **[7]**. Buckwheat flour (180 g) was soaked in deionized water solution (pH 9, 1:6, w/v) in a 40 °C water bath for 16 h. The soaked mass was removed of soaking solution before added of six times volume deionized water, and then milled with colloid mill instrument. The slurry obtained was screened through an 200-mesh (74 μm) sieve to collect the water solution, which was settled at 4 °C for 16 h. The supernatant and the top yellow protein layer were removed. The sediment layer was resuspended in deionized water and allowed to settle again centrifuged (3000×g) for 16min, and the yellow protein layer was removed again. This treatment was repeated third times. The sedimented starch was again resuspended in deionized water, adjusted to pH 6.5 with 1 M HCl, and centrifuged (3000×g) for 16min. The solid starch was collected, washed with deionized water for three times and dried at 45 °C with a vacuum drier for 10 h. After drying, the starch cake was powdered and passed through an 80-mesh sieve to obtain isolated tartary buckwheat starch.

Preparation of Tartary Starch Microsphere

Starch microspheres from tartary buckwheat starch were synthesized by inverse microemulsion technology as follows.

Preparation of oil phase: Required amount of Span 60 was added to 80ml of liquid paraffin under mechanical stirring at 55 °C until the Span60 was dissolved, which was incubated in constant water bath at 55 °C for further use.

Preparation of aqueous phase: a measured amount of starch was dispersed in 20 ml of deionized water solution which had been adjusted to pH 12 with 2 M sodium hydroxide beforehand. The solution was then stirred at 70 °C in constant water bath for 30 min before cooled to 40 °C for use.

Preparation of tartary starch microsphere **[1]**: The aqueous phase was added drop-wise slowly to the oil phase under mechanical stirring for 30 min for achieving uniform inverse emulsion disperses system. Cross-linking agent epichlorohydrin (3 ml) was then added to the emulsion, which was again stirred at 55 °C in constant water bath for another 4 h. After it was let stand for 10 min, upper oil layer was discarded, lower solid layer was washed in turn with ethyl acetate, absolute alcohol, and acetone, and was dried at 45 °C for 8 h a vacuum drier to obtain tartary starch microsphere.

Infrared Spectrum Analysis of Starch Microsphere

The Fourier-transform infrared spectrum (FTIR) of starch and starch microsphere were recorded on a Nicolet Nexus FT-IR spectrometer (Nicolet Instrument Co. U.S.A) using a potassium bromide disk, with the scanned wave number ranging from 4000 cm^{-1} to 400 cm^{-1}.

Morphological Characterization of Starch Microsphere

The morphology of starch and starch microsphere were observed in a S440 stereoscopic SEM (England, Leica Cambridge LTD) operating at 25 kV after they were coated with gold in small ions sputtering equipment.

Particle Size and Size Distribution Analysis of Starch Microsphere

The dry microspheres were dispersed in absolute alcohol, the particle size and particle size distribution were accessed by laser diffraction technique on a Mastersizer 2000 laser paricle analyzer (Malvern Instruments Ltd. Malvern, UK). The results were expressed in micrometers.

Adsorption Performance of Starch Microsphere for Methylene, Carminum and Lemon Yellow

The adsorption experiments were carried out in a series of conical flasks equipped with stoppers containing 0.2 g of starch microsphere and 25 ml of methylene blue, carminum or lemon yellow solution respectively at 37°C in a water rocker with constant stirring. The drug concentration before and after adsorption (within the fixed time) were determined respectively at wavelength 665 nm(methylene), 507 nm(carminum) or 426 nm (lemon yellow) by an TU1900 ultraviolet visible Spectrometer (Bingjing Purkinje General instrument Co, Ltd). The adsorbed amount of methylene on microspheres was computed as follows:

$$Q = \frac{V\,(C_o - C_e)}{m} \qquad (1)$$

Where Q, C_0, C_e, V, and m was the adsorption amount of drug (mg/g), the initial and equilibrium concentration of drug (mg/L), the solution volume (L), and the weight of starch microsphere, respectively.

Isothermal Adsorption Property of Methylene, Carminum and Lemon Yellow on Starch Microsphere

A group of drugs with different initial concentrations were incubated at 37 °C in a water rocker with constant stirring until the reaction . The equilibrium concentrations of drugs were mensurated respectively and calculated the Q according to the formula (1). The Langmuir isotherm model was used to study the adsorption isotherm properties of methylene, carminum and lemon yellow.

Langmuir Equation: $C_e/Q_e = C_e/Q_m + 1/(Q_m b) \qquad (2)$

drug (mg/g), b is adsorption equilibrium constant, Q_e is the adsorption amount of drug (mg/g) at equilibrium concentration of drug (mg/g).

RESULTS AND DISCUSSION

Morphology characteristics of starch microsphere

The prepared starch and starch microspheres were grey and free-flowing. **Figure 1** exhibits the morphology characteristics of starch and starch microsphere. The starch particles were polygonal or ellipse in shape and the former was in the majority. Starch microsphere indicated rough surface and was around ball shape. It had uniform particle size distribution. Some eyelets distributes on the surface of starch microsphere and formed porous structure, which would contribute to the adsorption of starch microsphere for other medicine and could be used as drug carrier or scaffold material for constructing of tissue engineering.

Structural Characterizations of Starch Microsphere by FTIR

The structural variation and characteristics of starch and starch microsphere were further explored by FTIR technology and the results are shown in **Figure 3**. The strong absorption band at 3399 cm^{-1} in native starch was assigned to stretching vibrations of the O-H which was characteristic peak of polymeric association and C-H at 1645 cm^{-1}, 858 cm^{-1}. Starch microsphere starch also exhibited O-H stretching at 3400 cm^{-1}, C-H stretching at 2929 & 1456 cm^{-1}. This means that hydroxyl groups always exist before and after emulsification and crosslinking process, but it is clear that the band in starch microsphere is less broad and much weaker than that in initial starch. In addition, it has been shown a weak C-O stretching at 1014 cm^{-1}, which is characteristic of ether functional moiety **[8]**, which means that crosslinking reaction between starch and epichlorohydrin has been occurred.

Particle Size and Size Distribution of Starch Microsphere

Particle size and size distribution are the most important characteristics of microsphere systems. They can influence the drug loading, drug release and stability of them. As shown in **Figure 3**, size distribution of both starch and starch microsphere almost took on normal distribution, and not presented two peak values or wider size distribution. The size distribution of starch varied from 2-15 μm with an average diameter of 5 μm. The size distribution of starch microsphere was in the range of 20-75 μm with an average diameter of 32 μm, and the diameters of them over 95% were smaller than 70 μm. This may be ascribed to the emulsification and crosslinking process, which is beneficial to the aggregation and coalescence and formation of microsphere with narrow and reproducible particle size.

The Adsorption Kinetic Characteristics of Three Dyes on Starch Microspheres

Adsorption time and temperature are the key factors affecting adsorption capability of starch microsphere. In our lab, we have found adsorption capacity of starch microsphere on methylene and carminum and lemon was the best at 40 ºC. All of them increased first and then decreased with the temperature in the range of 25 ºC -55 ºC (data not shown) owing to the wexothermic reaction in adsorption process. Allow for the application of starch microsphere in organism, the control temperature was 37 ºC in later experiments. To better understand the adsorption behavior of starch microsphere for methylene, carminum and lemon, the adsorbed amounts of them on ASMs were measured as a function of time respectively. As seen from **Figure 4**, three kinds of drugs were rapidly adsorbed by starch microsphere 60 minutes before. After it, the adsorption speed gradually reduced and the adsorption processes reached equilibrium in about 100 min. The mechanism for this phenomenon may be as follows. Methylene (carminum or lemon) was firstly adsorbed on the external surface of starch microsphere, and then the concentrations of them were decreased with the adsorption process going on. At this time, diffusion was the main control factor for adsorption rate which resulted in the slower adsorption rate. In the later period of adsorption process, adsorption action was mainly occurred on the internal surface. Adsorption process achieved equilibrium when the concentration difference and driving force gradually diminished with the reduction of drugs. It was worthwhile to note that the adsorption amount of carminum on starch microsphere was much higher than those of methylene and lemon.

For exploring adsorption mechanism such as mass transfer and physical-chemical reaction etc, different kinetic equations were used to fit the kinetic data (drug initial concentration, 40mg/L; 37 ºC). Elovich equation, parabola diffusion equation, pseudo-first-order rate equation and pseudo-second-order rate equation can be induced by drawing graph with Q_t versus lnt, Q_t versus $t^{1/2}$, $\log(Q_e-Q_t)$ versus t, and t/Q_t versus t, respectively. The results of fitting adsorption dynamics equations for different concentrations of drug are summarized in **Table 1**.

Elovich equation: $$Q_t=a+K_b\, t^{1/2} \quad (4)$$

parabola diffusion equation: $$Q_t=(1/\beta)\ln(\alpha\beta)+(1/\beta)\ln t \quad (5)$$

pseudo-first-order rate equation: $$\log(Q_e-Q_t)=\log Q_e-(k_1/2.303)\, t \quad (6)$$

pseudo-second-order rate equation: $$t/Q_t= 1/(k_2Q_e^2) +(1/Q_e)\, t \quad (7)$$

Where Q_t and Q_e were the equilibrium adsorption amount of drug (mg/g) respectively, K_1, K_2 and K_b were the first order adsorption rate constant, second order adsorption rate constant and internal diffusion rate constant ((g/（mg·min)), respectively. α and β were the initial adsorption rate constant and desorption rate constant respectively.

It can be seen from **Tables 1 a and b** that correlation coefficient R of second-order adsorption rate equation in four models was the biggest and ranged from 0.995 to 0.999. Second-order adsorption rate equation was the best model to describe the adsorption kinetics of methylene, carminum and lemon yellow on starch microsphere, followed by Elovich equation (R, 0.937-0.992), first-order rate equation (R, 0.926-0.973), and parabola diffusion equation (R, 0.861-0.984). When the concentration of methylene, carminum and lemon yellow were 20 g/L, the actual adsorption amounts of starch microsphere for them were 2.429, 1.803 and 1.413 respectively. However, the calculated adsorption amounts for them were 1.355, 1.042, and 0.933 according to first-order rate equation, 2.590, 1.923, and 1.647 by second-order rate equation. The values calculated from second-order rate equation were much closer to experimental values, which further indicated that second-order rate reaction kinetics model could preferably describe adsorption behaviors of starch microsphere for three dyes. For methylene and carminum, the correlation coefficients of Elovich equation and first-order rate equation also high. They were higher than 0.925. But the correlation coefficients of parabola diffusion equation were relatively lower and considerably changed with the increase of concentration. Thus, the adsorption process of mthylene and carminum on starch microsphere may include chemical adsorption and physical surface diffusion. As far as lemon yellow was concerned, correlation coefficients of four kinds of kinetics equations were higher and bigger than 0. 929, which suggested that the adsorption behavior also included internal diffusion except for chemical reaction adsorption and surface physical diffusion **[9-11]**.

The adsorption thermodynamics characteristics of three dyes on starch microspheres

Langmuir equation provides that the adsorption of adsorbens for dyes are monomolecular layer adsorption and there are no interaction between dyes molecules. As seen from **Table 2**, R^2 values of Langmuir equation for three dyes were 0.995, 0.992 and 0.994, which suggested that the adsorption property of starch microsphere for three dyes were physical adsorption modes.

Table 1 a: Regression Parameter of Starch Microspheres Adsorbing Methylene and Carminum and Lemon Yellow Kinetics Curve

Q_0 (g/L)	Parabola Diffusion Equation (Q_t=a+K_b $t^{1/2}$)			Elovich Equation (Q_t=(1/β)ln (αβ)+(1/β)） lnt)			
	R^2	K_d	Equation	R^2	β	α	Equation
20(CA)	0.928	0.430	Y=0.430X+1.081	0.973	3.968	1.790	Y=0.252X+0.494
40 (CA)	0.984	0.407	Y=0.407X+1.181	0.980	4.292	3.825	Y=0.233X+0.652
60 (CA)	0.861	0.578	Y=0.578X+1.163	0.937	2.899	0.938	Y=0.345X+0.345
10 (MB)	0.882	0.388	Y=0.388X+1.006	0.950	3.058	20.239	Y=0.327X+1.349
20 (MB)	0.953	0.358	Y=0.358X+1.606	0.984	3.390	204.017	Y=0.295X+1.929
40 (MB)	0.892	0.259	Y=0.259X+2.422	0.958	4.587	41851.269	Y=0.218X+2.652
10 (LY)	0.944	0.482	Y=0.482X+0.333	0.986	3.546	0.090	Y=0.282X-0.3231
20 (LY)	0.952	0.566	Y=0.566X+0.430	0.992	3.086	0.126	Y=0.324X-0.307
40 (LY)	0.929	0.661	Y=0.661X+0.562	0.972	2.584	0.162	Y=0.387X-0.337

Table 1 b: Correlation Coefficient R of Second-Order

Q_0 (g/L)	$Q_{e(exp)}$	first-order rate equation $\log(Q_e-Q_t)=\log Q_e-(k_1/2.303)\,t$				second-order rate equation $t/Q_t= 1/(k_2Q_e^2)+(1/Q_e)\,t$			
		equation	R^2	K_1	$Q_{e(cal)}$	equation	R^2	K_2	$Q_{e(cal)}$
20(CA)	1.803	Y=-0.591X+0.042	0.926	1.361	1.191	Y=0.520X+0.123	0.999	2.198	1.923
40(CA)	1.906	Y=-0.473X+0.018	0.932	1.090	1.042	Y=0.499X+0.119	0.997	2.092	2.004
60(CA)	2.102	Y=-0.669X+0.168	0.939	1.541	1.472	Y=0.44X+0.113	0.999	1.714	2.272
10(MB)	1.879	Y=-0.355X+0.114	0.992	0.813	1.294	Y=0.484X+0.267	0.999	0.877	2.066
20(MB)	2.429	Y=-0.346X+0.133	0.973	0.795	1.355	Y=0.386X+0.151	0.999	0.987	2.590
40(MB)	3.006	Y=-0.310X-0.132	0.925	1.175	1.842	Y=0.322X+0.06	0.999	1.729	3.105
10(LY)	1.135	y=-0.484X-0.353	0.980	1.114	0.885	Y=0.743X+0.345	0.998	1.269	1.345
20(LY)	1.413	y=-0.367X-0.031	0.979	0.845	0.933	Y=0.607X+0.364	0.997	1.012	1.647
40(LY)	1.687	y=-0.360X-0.015	0.951	0.829	0.966	Y=0.517X+0.269	0.995	0.994	1.934

Table 2 Parameters of Langmuir Equations of Drugs On Starch Microsphere

Drugs	Langmuir equation		
	Q_m /mg·g^{-1}	b/L·mg^{-1}	R^2
methylene	3.704	0.329	0.995
carminum	2.304	0.724	0.992
lemon yellow	2.128	1.500	0.994

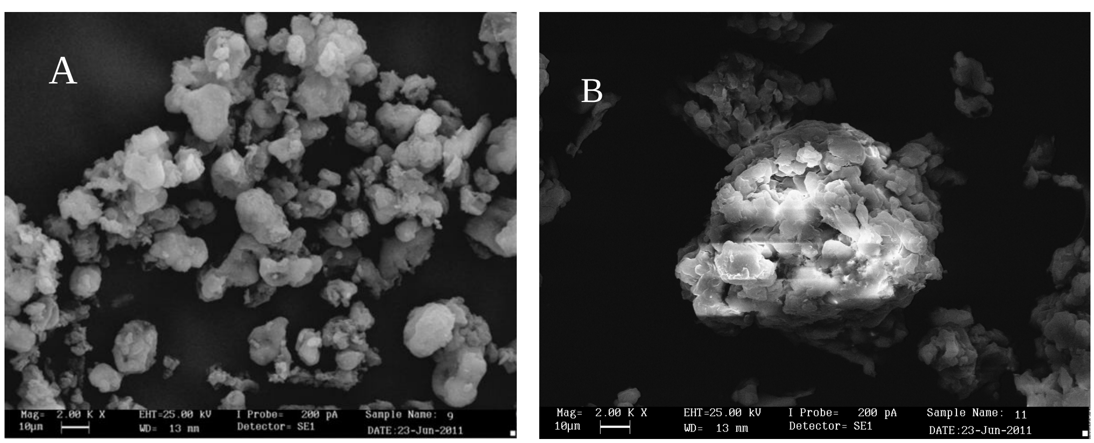

Figure 1: Scanning Electron Micrograph of Starch (A) and Starch Microsphere (B)

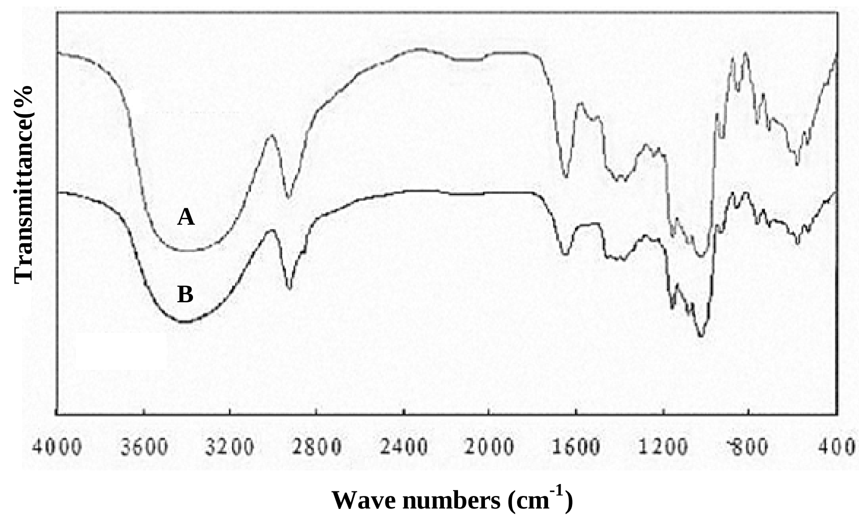

Figure 2: FTIR Spectra of Starch (A) and Starch Microsphere (B)

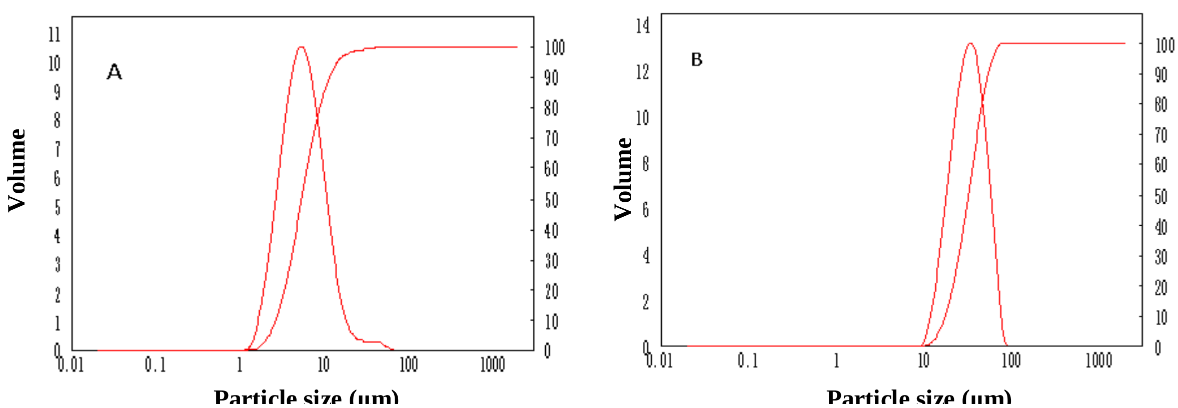

Figure 3: Particle Diameter Distribution of Buckwheat Starch (A) and Starch Microsphere (B)

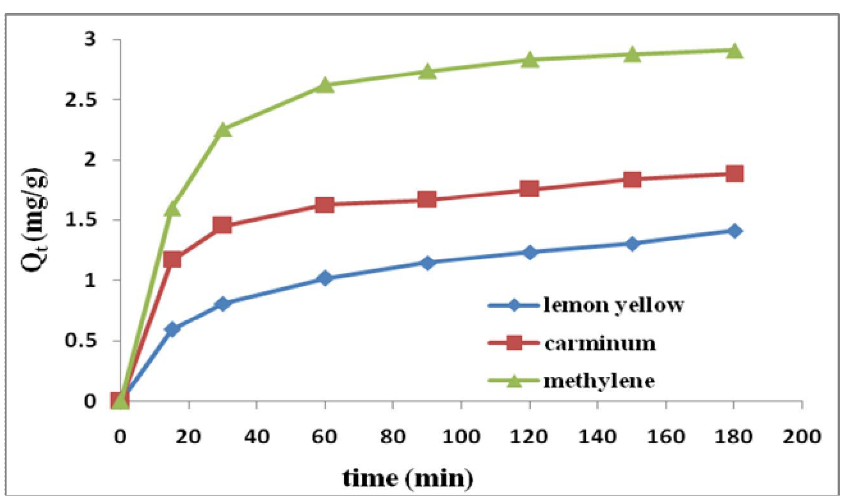

Figure 4: Adsorption Kinetic Curves of Methylene, Carminum and Lemon Yellow on Starch Microsphere C_0 = 40 mg/L

CONCLUSION

Tartary buckwheat starch microsphere was obtained by emulsification-crosslinking technology. It was around ball shape, exhibited rough surface had uniform particle size distribution. The size distribution of starch microsphere was in the range of 20-75 μm with an average diameter of 32 μm, and the diameters of them over 95% were smaller than 70 μm. Three kinds of dyes were rapidly adsorbed by starch microsphere 60 minutes before and the adsorption speed gradually reduced and the adsorption processes reached equilibrium in about 100 min. The adsorption equilibrium data of three dyes correlated well with the Langmuir isotherm model and fitted well pseudo-second-order kinetic model.

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