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Study of a Centrifugal Extractor for Protein Extraction Using Reversed Micellar Solutions

QIANG LU,* KUANHONG LI, MING ZHANG, and YAJUN SHI
CHEMICAL ENGINEERING RESEARCH CENTER
EAST CHINA UNIVERSITY OF SCIENCE AND TECHNOLOGY
SHANGHAI 200237, PEOPLE'S REPUBLIC OF CHINA

ABSTRACT

Based upon the characteristics of reversed micellar solutions, a cylindrical bowl centrifugal extractor Model HL-20 was tested for the extraction of protein. The hydraulic characteristics of this installation were investigated, and the optimal operation range was found. The interfacial radius in the rotating bowl was calculated, and the maximum throughput was determined from the dispersion number. The entrainment in the raffinate is lower than 5%, and the entrainment in the extract is about zero. The stage efficiency is above 95% under appropriate conditions. All of these verified that the cylindrical bowl centrifugal extractor is applicable to protein extraction by reversed micellar solution.

INTRODUCTION

With the development of the recombinant DNA technique and genetic engineering in recent years, the large-scale recovery of proteins and other bioproducts from fermentation and cell culture media has been given new emphasis. Liquid–liquid extraction of biomolecules using reversed micelles is a promising method when the traditional techniques with organic solvents are limited by protein denaturation and solubilization (1, 2). It has the advantage of high process capacity, operation on a continuous basis, and easy scale-up. Many properties of the water core of reversed micelles resemble those of water present at interfaces in biological systems, thus proteins can be

* To whom correspondence should be addressed.

hosted in the water core without loss of activity. The selective separation of a synthetic protein mixture (3), the concentration of α-amylase by continuous extraction using a 2-stage mixer-settler unit (4), purification of intracellular enzymes from whole bacterial cells (5), and recovery of an extracellular alkaline protease from an untreated fermentation broth (6) have been some of the successful applications of the use of reversed micellar systems on protein isolation and purification.

However, most works reporting on protein extraction by reversed micelles are focused on the distribution of proteins and performed in a batch mode, and little consideration has been given to the process itself and the selection of equipment. Based on the physicochemical properties of the extraction system and the process requirements, it is very important to research and develop a suitable apparatus on technique and economy. Although the extraction distributions are related to the chemical characteristics and thermodynamics of the system, equipment selection and development depend mainly on physical aspects and the kinetics. For reversed micellar systems the use of common extraction equipment might be limited by emulsion formation between the aqueous phase and the reversed micellar phase because of the presence of surfactants that stabilize those emulsions. Moreover, in order to maintain the conformation of proteins, the residence time of feed in apparatus should be as short as possible. Therefore, a centrifugal extractor may be a more suitable apparatus for liquid–liquid extraction of proteins using reversed micelles.

In this paper we first describe the construction and operation of the centrifugal extractor Model HL-20, including calculation of the interfacial radius in the rotating bowl. We then determine the maximum throughput from the dispersion number. Finally, we discuss its feasibility and operation range.

EXPERIMENTAL

Materials

Cetyl trimethyl ammonium bromide (CTAB) as a cationic surfactant was obtained from Beijing Xizhong Chemicals Factory, 99% purity, and used without further purification. Bovine serum albumin (BSA, molecular weight 68,000 Da, isoelectric point 4.9), a product of Sigma Chemical Co. Ltd., was used as received. All other (bio)chemicals were of analytical or chemical grade. The organic phase (reversed micellar solution) contained 20 mM CTAB in octane/hexanol (4/1) (v/v). Twenty vol% hexanol was added as a cosolvent (surfactant) to promote formation of a two-phase system. The density of the reversed micellar solution was 725 kg/m^3 at 25°C, and the solution viscosity was 1.07 mPa·s. BSA (1 kg/m^3) was dissolved in 0.01 M Tris–HCl buffer (pH 9.17) containing 0.1 M KCl for the adjustment of ionic strength.

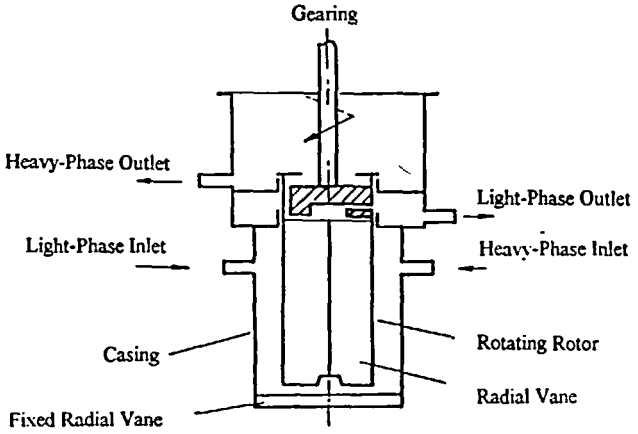

FIG. 1 Schematic configuration of HL centrifugal extractor.

For this aqueous protein solution the density was 997 kg/m^3 and viscosity was 0.903 mPa·s. The measured interfacial tension of this two-phase system was 1.5 mN/m.

Apparatus

The principal features of the experimental cylindrical bowl centrifugal extractor Model HL-20 are shown in Fig. 1. It consists of three sections: the gearing, the casing, and the rotating rotor. The annulus between the casing and the rotating rotor is a mixing zone. The inside of the rotor is a separating zone. The inside and outside diameters of the rotor are 20 and 23 mm, respectively. The height of settling zone is 40 mm. The inside diameter of the casing is 34.8 mm.

The accessory instruments are storage tanks, pumps, flowmeters, velocity meters, et al. They are not shown in Fig. 1.

Procedure

The power was first switched on and the motor was then started and set to a proper speed. When the rotor speed became constant, the light phase and the heavy phase were introduced. After steady-state had been attained, outlet samples of the two phases were taken out and the cotrainments in the two phases were measured. Light (heavy) phase cntrainment was determined from the volumetric percentage of the aqueous (organic) phase in the outlet light (heavy) phase. One minus the entrainment is the phase purity. The protein

concentrations of the inlet and outlet aqueous phases were also measured at 280 nm by using a Shimadzu UV-3000 spectrophotometer.

RESULTS AND DISCUSSION

Distribution of BSA

Previously we found that BSA can be easily dissolved in reversed micelles with CTAB as surfactant (7). Here, the distribution of BSA between the aqueous and the reversed micellar phase was studied in relation to the volume ratio of the two phases. The amount of BSA transferred toward the reversed micellar phase, as a function of the volume ratio, is given in Fig. 2. The results clearly indicate that the complete transfer of BSA from the aqueous solution to the light phase occurs for V_w/V_o up to 4. When the volume ratio is higher than this value, there is a loss of BSA that was not extracted from the aqueous solution. One of the reasons for this is that the loading capacity of the solvent is limited. Therefore, all of the following experiments were performed at a volume ratio less than 4.

Radius of Heavy Phase Weir and Interface in Rotating Bowl

The aqueous and organic phases are fed into the annulus between the contactor casing and the rotating rotor and are mixed by skin friction. The mixture flows down the annulus and then up through an orifice in the bottom of the rotor. The two phases are separated by centrifugal force as they flow upward through the rotor. Their clarifying liquid layers gradually widen, and the width

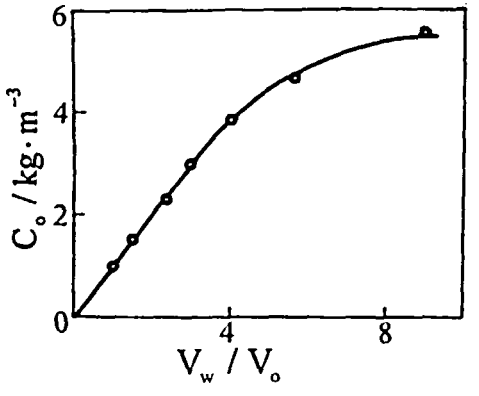

FIG. 2 Effect of aqueous/organic ratio on reversed extraction.

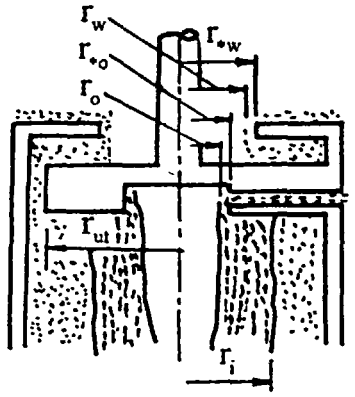

FIG. 3 Hydraulic characteristics of centrifugal extractor.

of the unseparated emulsion corresponding decreases. At the top of the rotor the emulsion band eventually vanishes, and the light–heavy phase interface is formed. This radius of interface (r_i) can be calculated from the overflow radius of the two phases (r_o and r_w) (Fig. 3) by using the principle that the pressures in the two liquid layers at the interface in a centrifugal force field are equal:

$$r_i = \sqrt{(r_w^2\rho_w - r_o^2\rho_o)/(\rho_w - \rho_o)} \tag{1}$$

where the overflow radius r_o, r_w is smaller than the radius r_{*o} (= 4 mm), r_{*w} of weir, respectively. Referring to the flow rate equation of a flat weir in a gravitational field, the flow rate equation of the annular weir in a centrifugal force field can be given by

$$r/r_* = \sqrt{1 - (q/Kf\pi\omega r_*^3)^{2/3}} \tag{2}$$

The fluid that overflows from a centrifugal force field will be subjected to Coriolis effects. As a result, the circular weir coefficients in a centrifugal force field (K) is different from that in a gravitational field (K_{**}). The relationship between them can be expressed by (8)

$$\ln(K/K_*) = (-4/bn)(K_*f\pi)^{2/3}(q/\omega)^{1/3} \tag{3}$$

where n is the number of radial vanes in the weir section. Both K_{*o} and K_{*w} are usually 0.33 (8). Meanwhile, the effects of both the flow resistance and potential energy increase must be included when r_i is calculated accurately. From Eqs. (1)–(3) it is shown that the weir radius of the heavy phase is the

only parameter needed to adjust the interfacial radius. Besides, the flow rates of the two phases will also influence r_i to some degree.

Before performing with the experimental system on the centrifugal extractor HL-20, we first tested the hydraulic characteristics by using heavy-phase weirs with five difference radius values ranging from 5.28 to 6.06 mm. There was serious entrainment at every flow rate tested. Hence, a heavy-phase weir with a radius of 6.31 mm was adopted, and a practical result was obtained. By knowing the construction parameters of the experimental apparatus, it is possible to calculate the interface radius using Eqs. (1)–(3) at any operating condition. The predicted results (Fig. 4) showed that the interface radius increased with decreasing total flow rate at a fixed flow ratio of the two phases, and it even exceeded the underflow radius (r_{uf}) of the heavy phase in the lower flow rate range.

Phase Purity and Operating Conditions

The position of the unseparated emulsion band (light–heavy phase interface) at the top of the rotor must be maintained between the weir radius (r_{*o}) of the light phase (near the center of the rotor) and the underflow radius (r_{uf}) of the heavy phase (near the periphery of the rotor) to prevent cross-contamination. For a heavy-phase weir with appropriate radius, increasing the flow rate of the light phase further would shift the interface outward, which could lead to entrainment in the heavy phase. On the contrary, increasing the flow rate of the heavy phase further would shift the interface inward, which might lead to entrainment of the light phase.

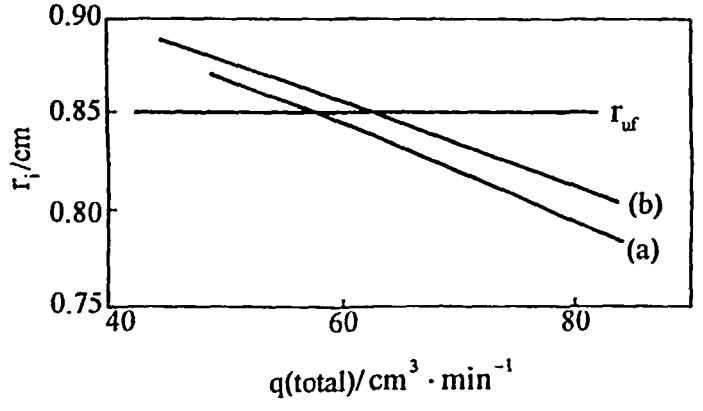

FIG. 4 Effect of total flow rate on interfacial radius. Flow ratio (O/A): (a) 0.25, (b) 0.33.

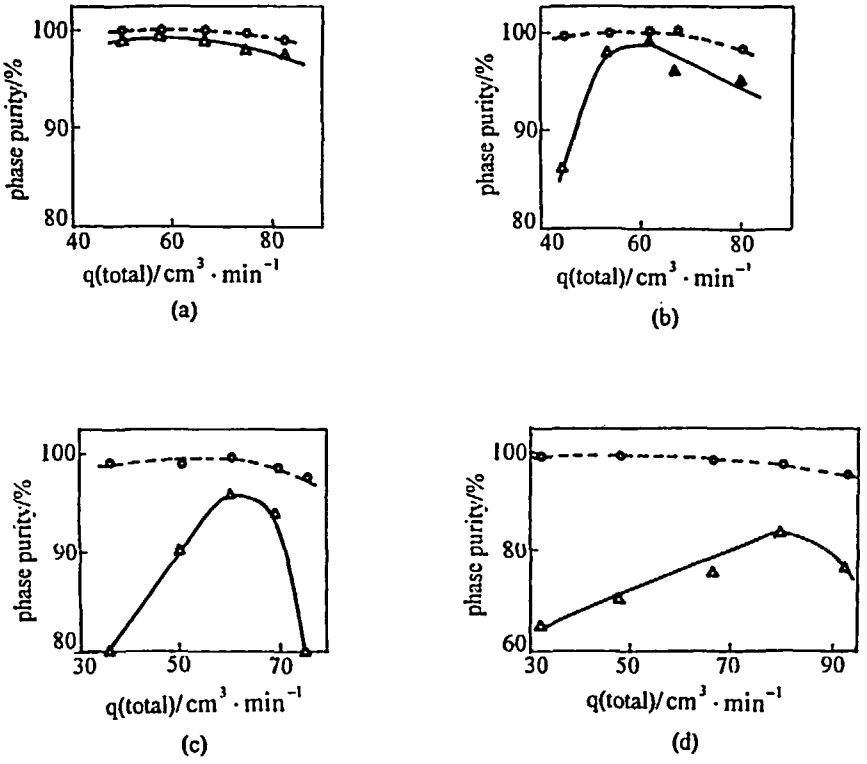

FIG. 5 Effect of total flow rate on phase purity. Rotor speed: 3000 r/min. Flow ratio (O/A): (a) 0.25, (b) 0.33, (c) 0.50, (d) 1.0. (Δ) Heavy phase purity, (O) light phase purity.

The dependence of the phase purity on total flow rate is shown in Fig. 5. It could be thought that the phase purity would decrease due to the separating load of the phases increasing as the total flow rate rises. On the other hand, the heavy-phase purity would increase due to the interface shifting inward as the total flow rate increases. Both effects cause the results shown in Fig. 5. It can also be seen that there is a total flow rate where the phase purity is a maximum at any flow ratio. Corresponding to this total flow rate, the flow rate of the heavy phase is almost the same, but that of the light phase varies with the flow ratio. Because the organic phase is very clear and the aqueous phase is somewhat cloudy at all flow ratios in our experiments, the light phase is regarded as a dispersed phase (9). Therefore, the results showed that the heavy-phase purity is lower.

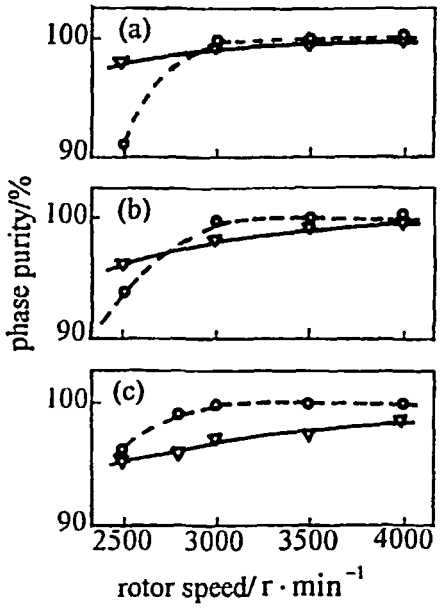

FIG. 6 Effect of rotor speed on phase purity. Total flow rate: 60 cm^3/min. Flow ratio (O/A): (a) 0.25, (b) 0.33, (c) 0.50. (▽) Heavy phase purity, (○) light phase purity.

The effect of rotor speed on the phase purity was measured at three flow ratios, as shown in Fig. 6. It is clear that there was a fairly high purity light phase with rotor speeds beyond 3000 r/min, and the purity of the heavy phase was improved by increasing the rotor speed or decreasing the flow ratio.

The Maximum Throughput and the Dispersion Number

Leonard et al. developed a dispersion number (N_{Di}) to characterize liquid–liquid dispersions produced in turbulent Couette flow with respect to their subsequent coalescence in a settling zone (9, 10). Moreover, a standard test was given to measure N_{Di} by using the following equation (9):

$$N_{Di} = \frac{1}{t_B}\sqrt{\frac{\Delta Z}{g}} \tag{4}$$

where t_B is the time for the dispersion to break and ΔZ refers to the initial thickness of the dispersion band. For the experimental system presently studied, a dispersion number of about 3.3×10^{-4} was measured by a gravity

batch test at three difference O/A values (0.25–0.5). Therefore, the solvent performance should be fairly good (9).

For a centrifugal extractor (10):

$$N_{Di} = \frac{q}{V_s}\sqrt{\frac{\Delta Z}{\bar{r}\omega^2}} \tag{5}$$

The average radius ($\bar{r}$) is given by

$$\bar{r} = \frac{2(r_{uf}^3 - r_{*o}^3)}{3(r_{uf}^2 - r_{*o}^2)} \tag{6}$$

The dispersion band will completely fill the separating zone at the maximum throughput, so the volume and thickness of the dispersion band can be determined. For the work reported here. $\omega = 314$ rad/s (3000 r/min), $V_s = 11.1$ cm^3 (see below), $\Delta Z = r_{uf} - r_{*o} = 0.45$ cm, and $\bar{r} = 0.652$ cm (using Eq. 6). Hence, the maximum throughput, $q = 83.1$ cm^3/min, was calculated from Eq. (5). By comparing this value with the experimental results in Fig. 5, it is concluded that the centrifugal extractor comes close to achieving the theoretical maximum throughput.

Stage Efficiency

The stage efficiency (η) is a comprehensive judge of the apparatus performance of mass transfer. Based on aqueous sample compositions, η was determined using the equation (11)

$$\eta = (C_{w,in} - C_{w,out})/(C_{w,in} - C_{w,e}) \tag{7}$$

where $C_{w,e}$ is the protein concentration in the aqueous effluent after equilibration with the organic effluent, and which is associated with the flow ratio. For the mass transfer conditions studied in this work, $C_{w,e}$ could be regarded as zero considering the partition of the protein in both phases.

Measurement of the protein concentrations in the aqueous phase before and after extraction gives the opportunity to calculate the stage efficiency as a function of the rotor speed at three flow ratios using Eq. (7). The results are shown in Fig. 7. Between the rotor speeds of 3000 and 4000 r/min, the stage efficiencies are fairly high and the curve shows a maximum at a rotor speed of 3500 r/min. This may be because mixing was not sufficient when the rotor speed was too low, whereas at high rotor speeds the residence time of feed in the mixing zone was reduced by increasing the rotor pumping capacity with increasing rotor speed. Both phenomena affect the mass transfer of the protein in the mixing zone.

The effect of a two-phase flow rate on the stage efficiency is complicated Perhaps the contact time of the two phases was not long enough for sufficient

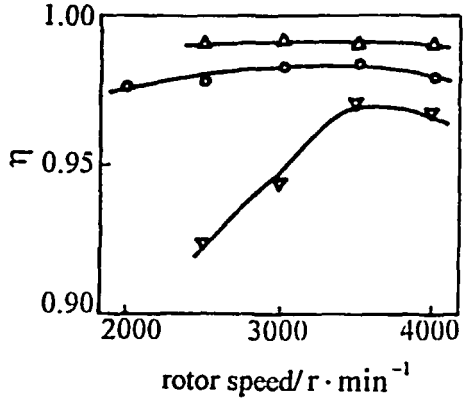

FIG. 7 Effect of rotor speed on stage efficiency. Total flow rate: 60 cm^3/min. Flow ratio (O/A): (▽) 0.25, (○) 0.33, (△) 0.50.

mixing to occur as the quantity of feed mixture increased to very high values, whereas as the total flow rate was decreased severely, the mixing of the two phases worsened, and the throughput of the apparatus was lower. The experimental results are illustrated in Fig. 8. The changes in the phase flow ratio will influence the interfacial behavior of the extraction system, and this will affect the mass transfer and the phases-separating performance. For example, the interfacial area between two phases becomes smaller as the flow

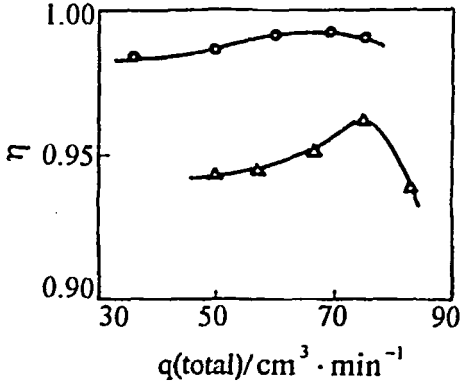

FIG. 8 Effect of total flow rate on stage efficiency. Rotor speed: 3000 r/min. Flow ratio (O/A): (△) 0.25, (○) 0.50.

ratio (O/A < 1) decreases at a constant total flow rate. Consequently, the stage efficiency becomes lower, as shown in Fig. 7.

Mixing and Phase Separating Time

According to the experimental results shown above, the optimum operation conditions for the system studied using the centrifugal extractor Model HL-20 were a rotor speed of 3000–3500 r/min and a total flow rate of 60–75 cm^3/min. The volume of the mixing chamber as calculated from the position of the inlets of the two phases was 12.4 cm^3. Considering an air core, the available separating volume inside of the rotor is given by (8)

$$V_s = \pi L(R^2 + gL/\omega^2 - r_{*o}^2) \tag{8}$$

hence

$$V_s = 11.1 \text{ cm}^3$$

If the lowest total flow rate was adopted, the mean time of mixing and phase separating are 12.4 and 11.1 seconds, respectively. The sum is 23.5 seconds; i.e., the mean residence time of feed in the apparatus is less than half a minute. Due to construction characteristics of the apparatus itself feed passes rapidly and uniformly through the mixing chamber and then inside the rotor. Thus, the stage efficiency is very high and hence this equipment is suited for the extraction of biomaterials.

CONCLUSION

The use of reversed micelles for the separation of proteins is a promising technique. A disadvantage of such systems is that the interfacial tension is lower, and that leads to the formation of rather strong macroemulsions. Moreover, the extraction of proteins must be operated at a short residence time. Therefore, a centrifugal extractor was selected as the most suitable equipment.

The protein BSA can be entirely extracted into a CTAB–hexanol–octane reversed micellar phase to a V_w/V_o up to 4, and the distribution data have been used to perform a continuous extraction of protein. A cylindrical bowl centrifugal extractor Model HL-20 was selected as the equipment for which a heavy-phase weir with fitting radius was made. A speed of 3000–3500 r/min, a flow ratio of 1/3–1/4, and a total flow rate of 50–75 cm^3/min were found to be the optimal operation range for which 95% heavy-phase purity, nearly 100% light-phase purity, and 95% stage efficiency for BSA could be obtained. The residence time of fluids in the equipment is less than 30 seconds. All of these tests demonstrated that centrifugal extraction of protein from an aqueous phase to a reversed micellar phase is possible.

NOTATION

b	thickness of liquid layer in weir section (cm)
c	protein concentration (kg/m^3)
f	utilization coefficient of weir periphery
K	circular weir coefficient in a centrifugal force field
K_*	circular weir coefficient in a gravitational field
L	height of settling zone (cm)
N_{Di}	dispersion number
n	number of radial vanes in weir section
q	flow rate (cm^3/min)
R	inside radius of the rotor (cm)
r	overflow radius (cm)
r_i	interfacial radius (cm)
r_*	weir radius (cm)
r_{uf}	underflow radius of heavy phase (cm)
$\bar{r}$	average radius
t_B	time for the dispersion to break
V_s	available separating volume (cm^3)
V_w/V_o	aqueous/organic volume ratio
ΔZ	thickness of the dispersion band
η	stage efficiency
ρ	density (kg/cm^3)
ω	angular velocity (rad/s)

Subscripts

in	flowing into extractor
out	flowing out of extractor
w	aqueous phase or heavy phase
o	organic phase or light phase

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