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Formulation and Development of Chitosan Based Microspheres of Glibenclamide

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ABSTRACT

The Microspheres are a well-thought-out controlled drug delivery method that can enhance the therapeutic efficacy of the medicine and address some of the problems associated with traditional treatment. As a medication delivery technology, microspheres offer a sustained and regulated pharmacological action in the targeted area of effect. The dosage and concentration of the medication should be chosen to maximize therapeutic effectiveness while reducing adverse effects. Chitosan is a flexible polymer that can be used in formulation research for anything from a medication carrier to a market weight supplement. Chitosan can be utilized to increase the bioavailability of low-soluble pharmaceuticals since it has been demonstrated to increase their rate of dissolution. The production of chitosan microspheres made extensive use of this cross-link.

It is safe, easy to use, and has numerous benefits over traditional ones, such as avoiding GI incompatibility, variable GI absorption, avoiding the first phase's metabolism, enhanced bioavailability, less frequent administration, more patient compliance, and rapid.

Keywords - Microspheres; Formulation; Development; Glibenclamide;Diabetes.

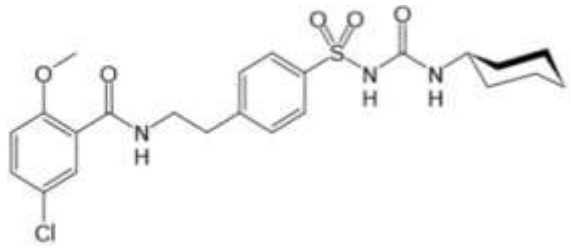
INTRODUCTION

The Diabetes mellitus is a chronic metabolic disorder with a high blood glucose level, frequently accompanied with insulin resistance. Patients with insulin-resistant Non-Insulin-Dependent Diabetes Mellitus (NIDDM) are treated with glibenclamide, an oral hypoglycemic drug. It is powerful but slow acting, noticeable early insulinameic activity, may function when other medicines fail. Hypoglycemia is more common; despite the short half-life, a single daily dose is possible. It is possible to maintain the controlled and sustained release plasma half-life by using this technique. In terms of output, encapsulative efficiency, particle size distribution, and in vitro release characteristics, chitosan-loaded microspheres for controlled release were developed and evaluated as promising and successful. The study of the best formulation revealed controlled drug release.

The medication that is supplied by encasing it in carriers like liposomes, microspheres, and nanoparticles, which modifies the drug's release and absorption (1-4). If customized to the target site, microparticular delivery systems are thought to be a reliable means of delivering the medication precisely and maintaining the required concentration in the site of interest without causing side effects (5). Micro-particles are sometimes referred to as micro-particles. They are made of waxy, polymeric, or other protective compounds.

The produced biodegradable polymers and modified natural materials like gums, proteins, lipids, waxes, and stulks. Albumin and gelatin are examples of natural polymers; synthetic polymers include polylactic acid and polyglycolic acid (6-8). Microspheres have a high volume-to-surface ratio and are very small. At the smaller end of their size range, they exhibit colloidal characteristics. The highly important interfacial characteristics of microspheres frequently determine their activity.

STRUCTURE OF GLIBENCLAMIDE.



MATERIAL AND METHODS.

Glibenclamide was received as a complimentary sample from Welcure Pharmaceuticals in Indore. Chitosan, alcohol from Sunchem India Pvt. Ltd., methanol, potassium dihydrogen phosphate, sodium chloride, n-octanol, acetone, and other materials were sourced from Rankem Lab. The analytical grade of the remaining chemical substances utilized was employed without any additional modifications.

PREFORMULATION STUDIES.

Identification, Solubility and characterization of drug sample:

Solubility Study of Drug: The saturation solubility method was used to determine glibenclamide's solubility. To create a saturated drug solution, the same amount of medication was added to test tubes that contained 10 milliliters of solvent. After filtering it via Whatman filter paper, the amount of medication dissolved was measured spectrophotometrically at 300 nm using a 1700 Shimadzu UV spectrophotometer.

Melting Point:

The melting point of the drug (glibenclamide) was determined by using capillary melting point apparatus and found in the range of 169-173 °C. Small amount of drug sample was taken in a capillary tube and the capillary tube was placed in a melting point apparatus and apparatus was switched on. Due to heat, the drug in the capillary tube was melted and the temperature was shown on the digital meter.

Partition Coefficient (Kp):

In a separating funnel, 10 mg of glibenclamide was precisely weighed and dissolved in 10 milliliters of phosphate buffer saline and 10 milliliters of n-octanol. This mixture was left for a full day after being shook for thirty seconds every ten minutes for an hour. A separating funnel was used to separate the two layers. Filter paper was used to filter the aqueous phase, which was then diluted 100 times with phosphate buffer saline. Using phosphate buffer saline as a blank in a 1700 Shimadzu UV spectrophotometer, the absorbance of the aqueous phase was measured at λmax 300 nm, and the concentration was calculated using the drug's standard curve.

Drug-Excipient Interactions Studies.

Fourier Transform Infrared Spectroscopy of Drug: The FTIR spectrum of glibenclamide should be compare with FTIR spectrum of mixture of glibenclamide and excipients used in the formulation and the-

re should be no interference in the peak of drug and excipients.

EVALUATION OF GLIBENCLAMIDE:

Preparation method- Gluteraldehyde was used as a crosslinker in the emulsification crosslinking procedure to create glibencliamide chitosan microspheres. A precisely measured amount of chitosan was dissolved in 2% (v/v) aqueous acetic acid. After adding the medication (glibencliamide), the chitosan solution was well combined. In order to create a water in oil (w/o) emulsion, the dispersed phase was then introduced dropwise via a disposable syringe (10 ml) to the continuous phase, which was made up of light and heavy liquid paraffin in a 1:1 ratio with varying concentrations of surfactant (span 80). A three-blade propeller stirrer was used to continue stirring at various speeds. A measured amount of aqueous gluteraldehyde (25%v/v) was added dropwise at regular intervals of 1, 2, and 3 hours, respectively, after 20 minutes of stirring.

Evaluation:

1. Shape Morphology: All batches of microspheres were studied for shape and size by optical microscopy at magnification of 1300X.
2. Percentage Yield (% Yield): The particle size analysis was done by optical microscope. Chitosan microspheres of glibenclamide (100 mg) was hydrated with PBS (pH 6.8) (10 ml) in a small test tube by manual shaking for 5 min. A drop of the suspension was mounted on the slide and observed under the optical microscope at 100 x magnification.
3. Particle Size: The particle size analysis was done by optical microscope. Chitosan microspheres of glibenclamide (100 mg) was hydrated with PBS (pH 6.8) (10 ml) in a small test tube by manual shaking for 5 min. A drop of the suspension was mounted on the slide and observed under the optical microscope at 100 x magnification.About 300 particles were measured individually with the help of eye piece micrometer, average was taken and their size distribution range, mean diameter was calculated.
4. Angle of Repose: The flow characteristics are measured by angle of repose. Improper flow is due to frictional forces between the particles. These forces are quantified by angle of repose. Determination of the repose angle was obtained for 10 g microspheres (23). For this study, microspheres were poured into a conical flask which had a 0.9 cm diameter and was placed 10 cm above the surface. Repose angle was calculated according to the tangent of the ratio of the height and diameter of the bulk. It can calculate by,

$\tan \theta = h / r$ or

$\theta = \tan^{-1} (h / r)$

Where, h =height of pile, r = radius of the base of the pile and θ = angle of repose.

5.Drug Entrapment Efficiency:

Weighed quantity of microspheres were crushed and suspended in methanol to extract the drug from microspheres. After 24 hrs, the filtrate was assayed spectrophotometically at 300 nm for drug content against methanol as blank. Corresponding drug concentrations in the samples were calculated from the calibration plot using a regression equation derived from the standard graph. The drug entrapment efficiency (DEE) was calculated by the equation.

Encapsulation efficiency = Actual drug content × 100 / Theoretical drug content

Swelling Index: The swelling ability of microspheres in physiological media was determined by optical microscopy method. The 100 microspheres were suspended in 5 mL of stimulated gastric fluid USP (pH

1.2). The particle size was monitored by microscopy technique every 1 hour up to 6 hours using an optical microscope. The increased size microsphere of each swollen sample (Wt) was determined by microscope.

Swelling index= (W2 - W1) × 100 / W1

W1 and W2 represent the final average partical size of chitosan microspheres and the initial average particle size of chitosan microspheres, respectively.

In-vitro Mucoadhesivity Test: The mucoadhesive property of microspheres was evaluated by in-vitro wash off test for mucoadhesion. A piece of mucosa was mounted onto glass slides. About 50 mg of microspheres were spread onto each wet rinsed tissue specimen and immediately thereafter the support was hung onto the arm of USP disintegration apparatus. By operating the disintegration test machine, the tissue specimen was given a regular up and down movement in

0.1 N HCl/ PBS pH 6.8 at 37°C taken in a 1000ml. At the end of 30 minutes, 1 hour and then at hourly intervals, the apparatus was stopped and the microspheres adhering to the tissue, 0.1NHCl/PBS was centrifuged, dried and the number of microspheres still adhering onto the tissue was counted. The mucoadhesiveness of these microspheres was calculated.

% Mucoadhesion = No. of microspheres remains × 100

/ No. of applied microspheres

***In-vitro* Release Study:**

The drug release was performed using USP dissolution (basket type) apparatus at 37 ± 0.5°C and at 50 rpm in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) as dissolution medium. Microspheres of 50 mg of glibenclamide were used for the test. Five milliliters of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, diluted suitably and analyzed spectrophotometricaly at 300nm using PBS pH7.4 as a blank. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of test sample.

RESULTS:

Solubility:

Table 1: Solubility of Glibenclamide in different solvent.

S.NO	SOLVENT	CONCENTRATION (MG/ML)	SOLUBILITY
1.	Water	5.45	Insoluble
2.	Methanol	36.08	Slightly
3.	Ethanol	45.75	soluble
4.	Chloroform	57.27	Slightly
5.	Dichloromethan	70.54	soluble
6.	0.1N NaOH	67.0	Sparingly
7.	Ether	5.59	soluble

Melting Point:

Table 2: Melting point determined by melting point apparatus

Sample no.	Melting point (°C)	average
1.	173	172°C

2.	170	
3.	173	

Partition Coefficient:

Table 3: Absorbance and concentration for determination of partition coefficient.

s.no	Solvent	absorbance	concentration
1	PBS pH7.4	0.233	5.1

Table 4: Partition coefficient of glibenclamide.

s.no	Solvent system	Partian coefficent
1	n-octanol : PBS pH 7	1.98

FTIR Spectrum:

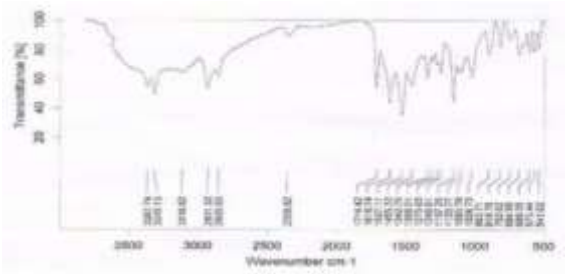


Fig. 2: FTIR spectrum of glibenclamide and chitosan.

Shape Morphology:

All batches of microspheres were studied for shape and size by optical microscopy at magnification of 1300X.

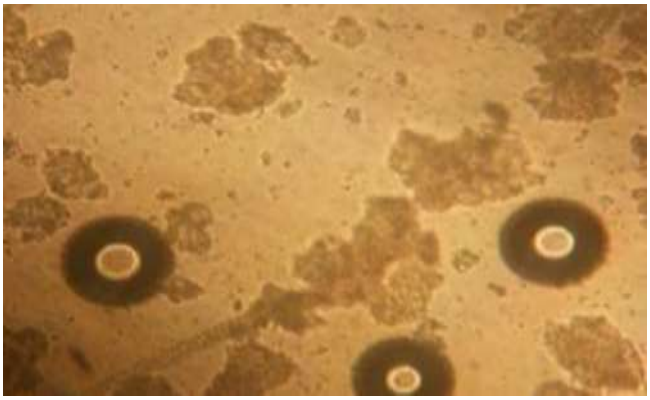


Fig. 3: Image of microspheres at magnification of 1300X.

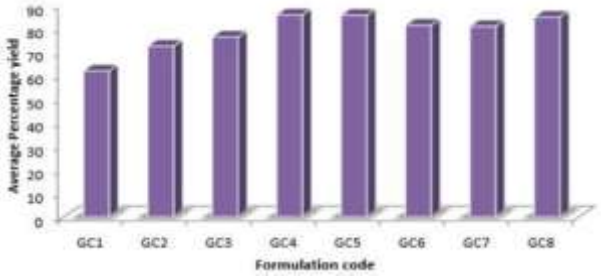
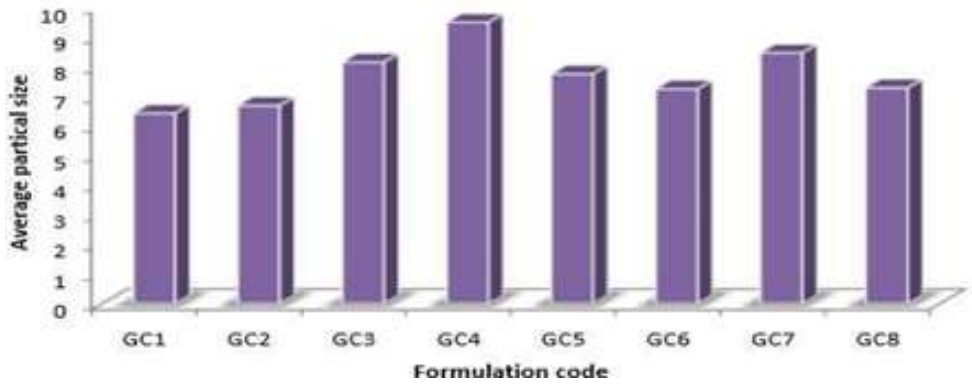


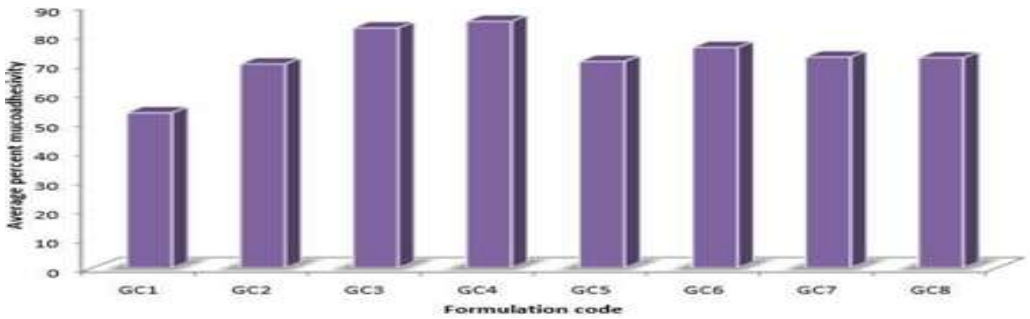
Fig. 4: Comparison graph of percentage yield of different formulations

Particle Size:

Table 7: Particle size analysis of different formulations

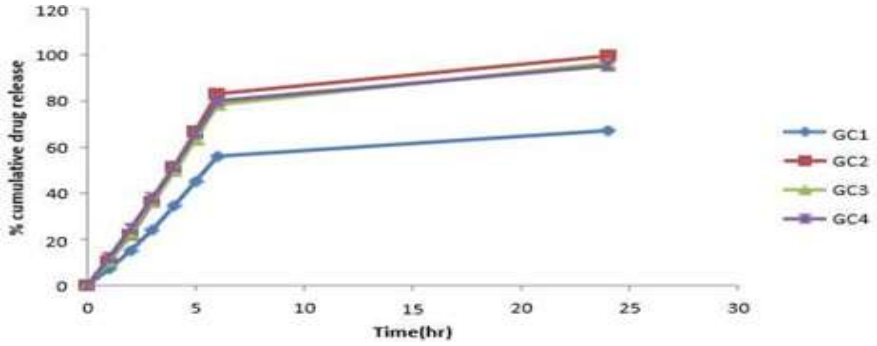
S. no.	Formulation	Average Particle
1.	Code	Size*± SD
2.	GC1	6.43±1.354
3.	GC2	6.69±1.50
4.	GC3	8.15±1.25
5.	GC4	9.48±1.74
6.	GC5	7.75±2.00
7.	GC6	7.24±1.54





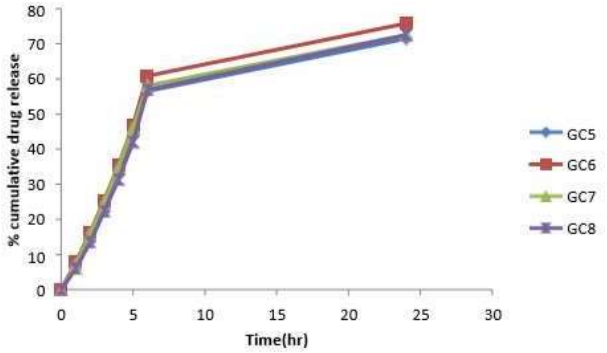
: Comparison graph of % Mucoadhesivity of different formulations.

: Comparison graph of %cumulative in-vitro drug release of 4 different formulations



In-vitro drug release study of formulation GC8

Time (hrs)	Time (hrs)	% Cumulative drug release
0	0	0
1	1	6
2	2	13.50
3	3	22.30
4	4	31.30
5	5	42.0



Comparison graph of %cumulative in-vitro drug release of 4 different formulations.

DISCUSSION:

The Glibenclamide, an oral hypoglycemic medication used to treat patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM) and insulin resistance, was chosen for this study. It has a significant initial insulinameic impact, is strong but slow-acting, and may be effective when other medications don't work. Hypoglycemia is more common, and a single daily dosage is feasible despite the short half-life. It is possible to preserve the plasma half life by using this technique, which is based on regulated and continuous release. Welcure Pharmaceutical, Indore (MP), provided the medication sample. Melting point A capillary tube melting point equipment was used to determine the drug's melting point. The melting point was determined to be between 169 and 173 °C, which matched the standard melting point listed in the pharmacopoeia. Thus, confirmthe identity of the compound. Partition coefficient Partition coefficient of the drug was determined in n-octanol: PBS. The value of partition coefficient was found to be 1.98. Higher the value of partition coefficient greater is the lipid solubility and vice versa. As the value of partition coefficient is found high, it shows that the drug is lipophilic in nature. Drug-excipient

compatibility Drug-excipients interaction was determined by FTIR spectrum it was found that the peak of glibenclamide has no interference with the peak of excipients. Hence there is no interaction between the drug sample and the excipients likely to be used in the formulation and hence can be used in the formulation.

Quantitative solubility of drug was checked in various solvent using 1700 Shimadzu UV spectrophotometer and found that the drug was slightly soluble in methanol, ethanol, sparingly soluble in dichloromethane, insoluble in water and water and dissolve in sodium hydroxide. From the studies performed it was concluded that Chitosan microspheres of glibenclamide can be prepared optimistically.via chitosan as a natural polymer, glibenclamide microspheres were created via a straightforward emulsification phase separation method. The polymer solution was made with 1% to 2% v/v acetic acid. The release rate was significantly impacted by the usage of different polymer concentrations. The ideal concentration was determined to be 2% v/v acetic acid, and the dispersion medium was 1:1 heavy and light paraffin.Due to its high rate of cross-linking and the fact that higher concentrations of glutaraldehyde produced strongly cross-linked spheres, a 25% v/v aqueous solution of glutaraldehyde was chosen as The chitosan microspheres are a useful to improve the uptake of hydrophilic substance across epithelial layer. The particle size of chitosan microspheres of glibenclamide was measured using an optical microscope with calibrated eyepiece micrometer. The mean particle sizes of all the microspheres formulations are in the range of 6.43-9.45µm. Vesicle size was found to be smallest in GC1 and particle size of GC4 was found to be the largest. The size distribution was found on the range of 1-6µm. It is noticed that the increase of the polymer ratio increases the mean particle size. With an increase in the drug polymer ratio from 1:1 to 1:4, bigger particles were generated as emulsion media viscosity was increased with an increase in polymer. As the viscosity increased, larger emulsion droplets were created that were harder to break and are therefore hasty, leading to an increase in the average particle size. It was found through the experiment that larger spherical microsphere were formed when the stirring speed was low (500 rpm). This could be owing to an inadequate speed of stirring that could not split the emulsion droplets. Removal speed beyond 2500 rpm resulted in the creation of tiny spherical microspheres, which might be at increased speed due to higher degrees of polymer phase emulsification. The mean particle size decreased significantly as the surfactant concentration increased. The medium concentration of span 80 is increased by the little amount due to the fact that droplet coalescence in the oil medium is not prevented. The mean particle size was lowered as the concentration climbed to 1.5%. A modest increase of the particle size of the formulation was also produced by the increase in the amount of crosslinking agent (gluteraldehyde). The adhesion of extra cross-linking agents on the microsphere surface may be responsible. The percentage yield was increases with increase the concentration of the polymer. This was due to the fact that with increase in chitosan concentration. The percentage yield was found to be higher with the formulation GC4 (85.66+0.577 %) and GC1 (62+1%) was found minimum. The value of Angle of repose of formulation within the range of 31°, indicating good flow properties for the microsphere. The entrapment efficiency was found to be higher with the formulation GC1(91.79+0.702) and entrapment efficiency of GC4 (76.20 +1%) was found to be least. Effect of crosslinking agent amount on entrapment efficiency was also studied. It was found that on increasing the amount of the crosslinking agent, the drug entrapment efficiency was decreased. To assess the mucoadhesive property of microspheres, In-vitro wash-off test was mediate. Formulation GC4 (84.64+2.38) showed the highest mucoadhesivity and GC1 showed minimum (53.3+2.08) mucoadhesivity. The swelling index was found

to be higher with the formulation GC4 (0.853+0.04) and of GC4 (0.556+0.06%) was found to be least. Microspheres swell in phosphate buffer.

6.8.Degree of swelling was found to be increased with polymer concentration and cross-linking time. The bioadhesive property of the microspheres also increased as swelling index increases. In-vitro release of chitosan microspheres of glibenclamide was done by dissolution apparatus. The highest % cumulative release was found in GC2 (99.62%) and lowest drug release is in GC1 (67.2%). Mathematical models are commonly used to predict the release mechanism and compare release profile. It was found that drug release rate decreased as the concentration of chitosan increased and also with increased cross-linking time.

CONCLUSION-

The Microspheres serve as a meticulously crafted controlled drug delivery system that can address certain challenges of traditional treatments and enhance the therapeutic effectiveness of medications. Microsphere as a drug delivery system ensures the targeted area of effect with sustained and controlled drug activity. The medication must be given at a rate and concentration that ens Microspheres are a well-designed controlled drug delivery device that can resolve some of the conventional treatment issues and improve the medication's therapeutic efficacy. Microsphere as a mechanism of drug delivery provides the intended area of effect with a sustainable and regulated activity of the drug. The medicine should be administered at a rate and concentration that allows for optimum therapeutic efficiency while minimising side-effectsures maximum therapeutic effectiveness while reducing side effects.Diabetes mellitus is a chronic metabolic condition with a high blood glucose level, commonly associated with insulin resistance. Glibenclamide is an oral hypoglycemic agent, which is a drug for the treatment of patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM) and given in insulin resistance condition. It is potent but slow acting, marked initial insulinameic action, may work when other drugs fail. Higher incidence of hypoglycemia, single daily dose possible despite short half-life. Using this system based on controlled and sustained release plasma half life can be maintained.In terms of output, encapsulative efficiency, particle size distribution, and in vitro release characteristics, chitosan-loaded microspheres for controlled release were developed and evaluated as promising and successful. Regulated medicine release was found in the optimal formulation experiment.

FUTURE ASPECTS

While the control of drug release profiles has been a major aim of pharmaceutical research and development in the past two decades, the control of GI transit profiles could be the focus point of researchand might result in the availability of new products with better therapeutic possibilities and substantial benefits for patients. Mucoadhesive microspheres would become the promising candidate for delivery various drugs in sustained release manner. Dosing frequency and loss of drug also reduced by use of such type of formulations. Thus Chitosan microspheres of glibclamide would become a promising candidate for therapy of diabetes type-II in the future

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