Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2018.0404.19**

A FASCINATING SELF-ASSEMBLY OF THE HONEY MEDIATED CURCUMIN–CASEIN NANOPARTICLES

Joly A[1,2], Latha M S[2,3*]

1. Sree Narayana College, Varkala, Thiruvananthapuram, Kerala, India.

2. Sree Narayana College, Chengannoor, Kerala, India.

3.Sree Narayana College, Kollam, Kerala, India.

ABSTRACT: Curcumin driven assembly in casein polymer in presence of 1:1 honey operates natural self-organization through noncovalent interactions and formed structures of very fascinating shape. Doughnut, rod, hexagonal, square and cube-shaped nanostructures, were self-assembled from curcumin-casein conjugate in presence of honey. We demonstrated that monodisperse nanocurcumin were formed inside the cavities of these structures on changing concentration as well as fluidity. These features may allow the nanocurcumin-casein has potential application in nanomedicine, drug delivery and many therauptic applications. Here we report nano and micro scale structures with well-defined sizes and shapes due to self-assembly. These resulting architectures formed are in definite shape and are mechanically stable.

KEYWORDS: Curcumin, Casein, Honey, Self-assembly, Nanostructures

Corresponding Author: Dr. Latha M S* Ph.D.

1.Sree Narayana College, Chengannoor, Kerala, India. 2.Sree Narayana College, Kollam, Kerala, India

Email Address: lathams2014@gmail.com

1.INTRODUCTION

Curcumin, the natural polyphenolic compound from Curcumin longa, has been commonly used as a traditional medicine [1]. Earlier reports show that, which has long been known for its anticancer, antioxidant, and anti-inflammatory activities [2]. Many studies have shown that curcumin, which is used as a dietary supplement has very high potential for its chemotherauptic effect [3]**.** Casein a biodegradable, biocompatible, non-toxic, and low cost polymer, which shows many interesting properties. Studied reports show that in milk caseins exists as a natural nanoform, which can be used as a carrier for hydrophobic drugs and the curcumin can form complex with bovine casein micelles (CMs). This can be used as a vehicle for drug delivery to cancer cells. DLS studies showed that CM suspension of 200nm was stable in buffer solution (pH 7.4). SEM and AFM studies gave the evidence that the particles were roughly spherical in shape. This study again confirmed the hydrophobic interaction of casein- micellar curcumin complex. A steady state fluorescence spectroscopy study reported the binding constant interaction of 1.48 × 104 M−1 casein micellar curcumin complex [4]. It was showed that curcumin-loaded casein nanoparticles were bigger in size which were processed at encapsulation conditions and the increased nanoparticle dimension from fluorescence and FTIR spectroscopy results, suggested that curcumin was entrapped in the nanoparticle core through hydrophobic interactions. The curcumin encapsulated in casein nanoparticles had higher biological activity, as assessed by antioxidant and cell proliferation assays, then pristine curcumin, lipophilic bioactive compounds likely due to the improved dispersibility [5]. Polymeric assembly causes the formation nano/micro structures with well-defined sizes and persistent structures [6][7][8][9] [10]and its topology plays a crucial role in defining the properties of the material in addition to the composition of the polymers. The topology can also give information about the surface characteristic for chemical modification, viscosity, binding or encapsulation [11]. Different structures have been formed from organic molecules and polymers through self-assembly in solution and self-organization on solvent evaporation on surface [10 -14] Here we reported the preparation of nano sized curcumin-casein polymer conjugate of different architectures involving shape directed covalent [15] and non-covalent assembly [16] or self-organization [17]. In this report the formation and assembly of molecules occurs at room temperature. The exhibited nano-assemblies, as observed through AFM, SEM, TEM, and DLS are uniform. Curcumin-Casein nanoparticle assembly shows a fascinating aggregation of nano casein with nano curcumin which may be due to non-covalent interaction [18-21]**.** Curcumin having a P^H 6.8 and casein a polymeric protein both in honey exert the driving force for molecular interaction. Driving forces may arise due to no bond interactions such as dipole-dipole, ion-dipole, π-π interaction of π-stacking, H-bonding, van der Waal's and electrostatics forces and causes self-assembly which are highly system specific. Recent findings have demonstrated casein polymer on appropriate treatments allow control of the shape, morphology, mechanical properties, porosity and surface area of. Extensive research has been carried out on curcumin around the world to demonstrate its great potential as a therapeutic agent and has shown the way towards clinical trials for a variety of diseases [22-25]. However, one of the greatest challenges in developing curcumin for clinical efficacy is its low oral bioavailability due to its hydrophobic nature. Curcumin enhances its bioavailability and hence improve the activity, absorption and metabolism within the living system and reduce the rapid elimination from the system by nanoencapsulation of casein in honey medium [26]. Here we tried to prepare curcumin-casein nanoparticles using honey as surfactant by normal and simple sonication method. The prepared nanoparticles were characterized by using DLS, SEM, AFM, and TEM. In addition, this study reports the chemistry on the surface morphology and size of the developed nanostructures curcumin –casein nanoparticles in honey [27]. We found that the size of the particles determined the partitioning phase without a change in the surface property of the particles. The observed the size-dependent uptake of particles in a cell. In further studies, it may be useful to investigate the dynamical behavior of nanoparticle. The different type of no bond interactions gives different fascinating structures for these particles which were confirmed by their SEM and AFM images. The bonding was again confirmed by FTIR, and fluorescence spectroscopy etc. The conjugate was developed to enhance the developed nanoparticle systems. The solubility and stability of curcumin in aqueous solution by self-aggregation to generate micelles with hydrophilic alginate in the outer shell. The alginate curcumin conjugate was treated with a surfactant to decrease the size of the particle in nano level. These particle size can be characterized by DLS, SEM & TEM.

2. MATERIALS AND METHODS

Curcumin (95% total curcuminoids content was obtained from Alfa Aesar (Bangalore, India) Casein was purchased from Merck. Surfactant is pure, natural and is purchased from Vellayani Agricultural College, Kerala, India. All other reagents were of analytical grade and used as received. Preparation of nanocurcumin-Casein Nanoparticles in Honey. The curcumin-casein -nanoparticles were prepared using the gelation technique. Briefly, an aqueous alginate solution (0.05 and 1% w/v) was prepared using 5mL of 0.01M acetic acid and made up to a 500mL with distilled water. The mixture was magnetically stirred for 24 hrs. and sonicated using a bath sonicator for 30 min at room temperature. After sonication, drop wise addition of the curcumin solution (0.005g of curcumin dissolved in 15mL of acetic acid) to casein solution and again stirred the mixture to obtain a fine nanoparticle suspension. The curcumin-loaded casein nanoparticle dispersion was then kept in room temperature by under stirring 48hrs.To the above final mixture 1:1honey (5mL with drop wise addition) was added with continuous stirring and again the whole mixture is sonicated for 30min.

Particle Size Measurements.

SEM

The size of nanoparticles was again confirmed by SEM analysis using NovaNanoSem NPEP252 immersion lens mode at 5.00Kv by gold sputtering on the material coated on a sheet of carbon paper at 210kV.The images of different shapes and sizes were obtained. The particles of size ranging from 50-300nm range were obtained

DLS

To determine average size and size distribution of Nanocurcumin, DLS analysis was performed The size of the curcumin- alginate nanoparticles was determined by DLS and its zeta potential is determined Zeta potential measurements of synthesized nanoparticles were performed with Zetasizer version 7.11 (Malvern Instrument ltd, UK) by using zeta dip cells. Zeta potential of synthesized nanoparticles was analyzed to determine the charges present on the surface of nanoparticles and its stability at pH 7.4. The samples for analysis were prepared by mixing of nano curcumin colloid in water in1:10 proportion. For measuring zeta potential, 1000 µl of the sample was taken in clear disposable zeta cells.

SEM

The size of nanoparticles was again confirmed by SEM analysis using NovaNanoSem NPEP252 immersion lens mode at 5.00Kv by gold sputtering on the material coated on a sheet of carbon paper at 210kV.The images of different shapes and sizes were obtained. The particles of size ranging from 50-300nm range were obtained.

AFM

Samples were prepared and coated on a silicon wafer. AFM images were collected on a digital instrument in tapping mode under ambient conditions Height profiles were obtained using nano scope software and also histogram is also plotted. Raw Mean -0.000254 nm: Image Mean -0.000254 nm: Image Z Range -0.685 µm image Surface Area -109 μm^2Image Projected Surface Area -100 μm^2: Image Surface Area Difference -8.57 %: Image Rq -0.119 µm: Image Ra -0.0984 µm Image Rmax -0.685 µm

TEM

TEM was useful to determine the size and topology of synthesized nanocurcumin-casein in honey. A drop of the solution containing nanocurcumin-casein was placed on the carbon coated copper grids and kept in an infrared light until sample gets dried. After drying powder of nanoparticles was loaded on specimen holder. TEM images also reveals the difference in shape of alginate-curcumin nanoparticles with a range of 20-100nm.

FTIR

Fourier transformer spectra were taken from an instrument model spectrum 400, cpu32 main 00.09.9932, strong ratio spectrum magnitude, double combined, using KBr pellet.

3. RESULTS AND DISCUSSION

Preparation and characterization of supramolecular nano assemblies of curcumin-casein complex in honey are of different sizes and structures. This enables the application of nano architectures in selective drug or biomolecule delivery for therapeutic purposes. The synthesis and characterization of Nanocurcumin-casein in honey were made at varying concentrations 20µg-100µg/ml by weight having a P^H6.8. All the nano assemblies were obtained at room temperature at 30- 32^0C.It exhibited multi supra molecular assemblies as observed in SEM, AFM, TEM. DLS measurement indicates the nature of self-assembly process. To understand the qualitative long range nanoscale behavior of the nanocurcumin in casein nanoparticles with honey as surfactant, microscopy method SEM is important for the system Samples imaged by SEM were of varying concentration. The images of different shapes and sizes were obtained. The particles of size ranging from 30-80nm range were obtained. The shapes are unexpected and fascinating as shown in figure2 (a)-(b)

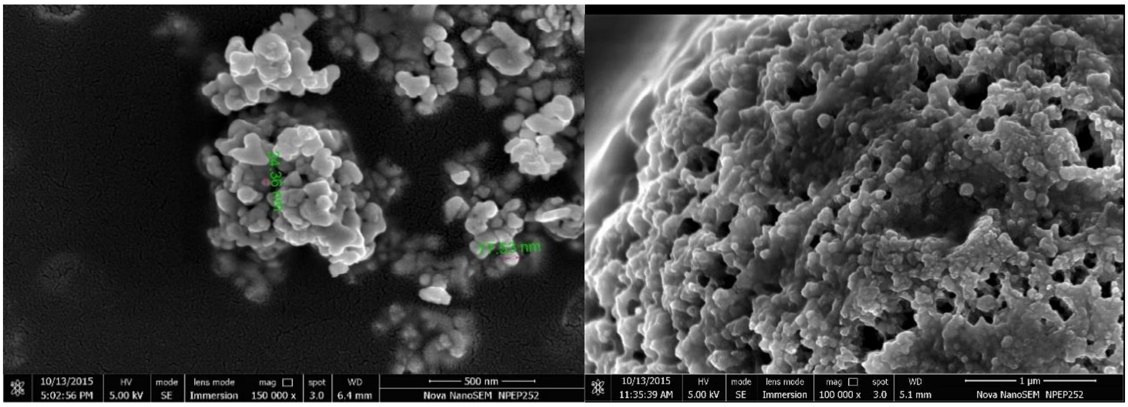

Figure 1a and b Microscopy SEM images with different nano architectural assembly's in 0.5% casein and1:1 honey

SEM images (figure1a-b) of the above fascinating nano level molecular architectural assemblies may be due to the supramolecular formation of nano curcumin with casein and honey. In the proposed study we only intended to prepare nanocurcumin preparation, characterization and its application as a chemotherapeutic agent. When we vary the concentration at constant pH, here we report unexpected finding of supra molecular assemblies due to the unexpected interaction between curcumin and casein in a controlled fashion in honey. Even in a well-controlled polymerization path way the chain length as well as the molecular architecture of the various individual present in a given medium are not the same. Curcumin and casein are hyper branched macromolecules to nanosized having very high surface area. From earlier studies it is clear that the most stable form of curcumin is bent shape which is the lowest energy structure of curcumin. Curcumin has phenolic and methoxyl in its phenyl ring and also these two phenyl ring are connected by a conjugated system with two c=o groups flanked by a -CH_2 group. But there exists a keto-enol tautomerism. This keto and enol form can form weak intermolecular H- bonding with alginate molecule. Supramolecular structures are the results of not only additive but also cooperative interactions, and their properties are generally follow from their supramolecular characteristics. Isotropic medium ranges intermolecular interactive forces such as van der Waal's forces, π-π interactions etc. define the shape as well as size and close packing of molecule. In the curcumin –casein nano assemblies in honey we have a number of such isotropic interactions because alginate have two carboxyl groups and six –OH groups. All these moieties can form inter molecular H-bonding, no bond interactions such as dipole-dipole, van der Waal's and π-bond interactions with two phenyl ring and the conjugated system of the curcumin. The uneven distribution of electrons in curcumin and casein can result a pair of partial charges and also polar covalent bond creates bond dipole inside the molecule. Another common dipole arises from lone pair of electrons from electro negative oxygen of hybrid orbital of curcumin and alginate. In curcumin a conjugated π- system is present and alginate has two cation moiety of carboxylates, therefore we can expect a cation –pi interaction which can be stronger than hydrogen bonding. Cation-pi interaction may occur between alginate cation and aromatic or double bond region of curcumin. The dough –nut shaped SEM images with a size range below 800- 500nm due to strong no bond interaction interactions at a higher concentration (50µg/ml curcumin in1% alginate). In these nano structures curcumin is in its most stable bent shape which is surrounded by an alginate polymeric ring at the center there exists an inward force towards curcumin located inside the dough –nut shaped nano architecture. This may be due to the strong π-π interaction in nanocurcumin. Figure 5b shows the rupturing of the organized assemblies when the attractive forces overcome the repulsive forces. Figure5e-g have superior level of architecture (like a papaya cut into two pieces). Such structural assemblies are seen as paired having nanoparticle in the range 170-600nm. It is a close packed and a regular arrangement which may appear to be stable. At another level of concentration range we have rod shaped assemblies figure h and I. It may be due to the parallel alignment curcumin with alginate. Particle size again reduces in these assemblies about150-370nm. This rod like structure allows good molecular contact to promote the one dimensional growth through monovalent interactions such as ion-ion, ion-dipole, dipole-diploe, H-bonding, π-π interaction and hydrophobic effect. Polarization of π-systems by hetero atom such as oxygen may lead to direct face to face geometry. Figure j and k shows nano-assemblies in another fashion with a size range of 150 200nm. Decreasing the particle size causes the increase in surface area which enables to increase the no bond interactions. Hence the shape also reduces. The SEM image figure l shows a close packed with definite geometrical structures cubes with 20.

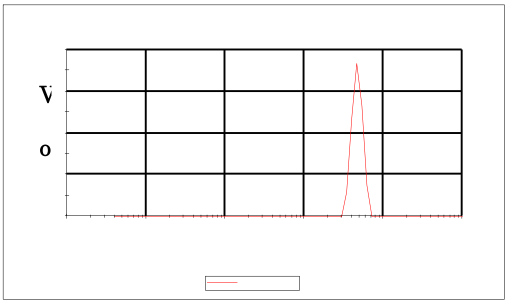

Figure 2 - DLS results having particle intensity maximum t 468n

Dynamic light scattering data (figure 9) reveals the shape formation of nano assemblies which give support for SEM images. In particle size measurements refractive index of the material was found to be 1.42 and the water having refractive index 1.33 was used as dispersant. The viscosity of the solution is very less having a value of 0.8872. m. Material absorption was found to be 0.02. From DLS measurements particle size intensity was maximum in the 300-500nm range. Optical density measurements with different time interval were noted for casein-curcumin in honey, casein-curcumin alone and curcumin only. The variation in optical density with change in concentration that is by varying concentration ofcasein from 0-14µl to a fixed concentration of 10 µl of curcumin and vice versa (that is by varying the concentration of curcumin from 0-14µl to a fixed concentration of 10µl ofcasein). The optical density shows steady increase after 1hr minutes for casein - curcumin nanoparticles in honey. The first 30 minutes all the three shows same increase up to a certain level. But in casein –curcumin there is a sudden decrease and remains constant. For plane nano curcumin a sudden increase occurs and attain a maximum value and then remains constant. This again confirms the structural aspects found in curcumin –Casen honey. Optical density graph with concentration variation also shows significant result. AFM also shows the versatility of supramolecular nanoassemblies which shows the following figures-3

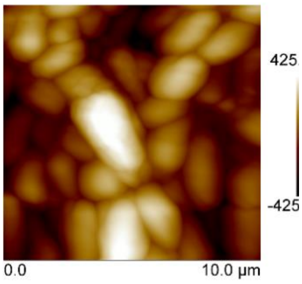

Figure 3 AFM images of nanoassemblies of curcumin-alginate nanoparticles in honey.

AFM also shows the versatility of supramolecular nano assemblies which shows the above figure-3. AFM images (figures 3) different surfaces will again confirmed by the nano assemblies in SEM images. AFM also like SEM show that some of the structures have either a partially or completely opened nano assemblies with increase in concentration. Well-ordered geometrical structures were obtained at low concentration. As the concentration increases the rate of aggregation becomes faster and as a result the defect sites occurs or rupturing takes place. But at lower concentration the assemblies will be an ordered fashion and precise geometrical structures are obtained. At the lowest concentration, feasible monomers self- organized to form a single size and stiff nano assemblies with the internal energy dominating the entropy. As concentration increases entropy also increase which causes deformation in structures. AFM images are not as expected as in SEM because of the flattening of surface forces or from the AFM tip itself.

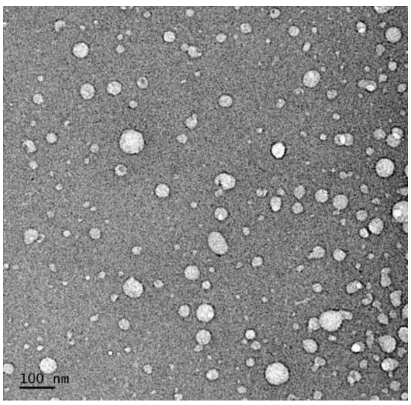

Figure 4 TEM Microscopy images of curcumin –casein in honey that show 100nm nano level molecular assembly

TEM is an integral technique used to view the morphology and length of these fascinating self-assemblies in nanoscale. For this 50µg/ml sample of curcumin in acetic acid is treated with 1ml of aqueous 1% casein and 1ml of 1:1 honey as described in the procedure. TEM images (Figure1). Figure-1 show stem images at 100nm particle size. From these we believe the presence of nano assemblies in curcumin-casein in honey.

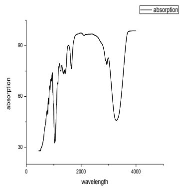

Figure 5: FTIR spectra:1curcumin-casein in honey

Analyzing using FTIR spectroscopy (figure5) the formation of nanoparticle and its hierarchical assemblies were revealed which is in good agreement with the SEM observation. Casein -curcumin conjugate has a lower O-H frequency (3340.34cm^{-1}) than curcumin comparing with the FTIR spectrum curcumin from literature. It is because in curcumin-casein conjugate polymeric assemblies' curcumin nanoparticles to natural casein nanoparticles are formed. C-Stretching frequencies is at 2112.16 cm^{-1} which is also lower than that of plane curcumin solution. The honey controls the aggregation of curcumin in a steady manner hence attain a regular shape and reduces the particle size there by increasing the surface area. This causes a very high hike in intermolecular interactions, which may cause the fascinating self-assemblies.

4. CONCLUSION

In this report the formation of self-assembling nanoparticles with fascinating architectures. Concentration variation shows the difference in assemblies of curcumin-casein nanoparticles in presence of honey. But in the absence of honey the nano assemblies are not well organized. We expect these well-organized structures are promising in the field of anti-cancer medicines and drug delivery.

5.CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Hasima N, Aggarwal BB. Targeting proteasomal pathways by dietary curcumin for cancer prevention and treatment: Cur. Med. Chem. 2014;21(14),1583-94. PMID: 23834173
2. Shishodia.S, Misra K, Aggarwal B B. Turmeric as cure-cumin: promises, problems, and solutions in Dietary modulation of cell signaling pathways: (eds. Surh, Y. J., Dong, Z., Cadenas, E. & Packer, L.) 91–99 (CRC Press, 2009).
3. Goel A, Kunnumakkara A B, Aggarwal B B. Curcumin as "Curcumin": from kitchen to clinic: Biochem. Pharmacol.2008;75:787–809.
4. Arbiser J L et al. Curcumin is an in vivo inhibitor of angiogenesis: Mol. Med. 4.1998; 376–383.
5. Abhishek Sahu, Naresh Kasoju, and Utpal Bora, Fluorescence Study of the Curcumin−Casein Micelle Complexation and Its Application as a Drug Nanocarrier to Cancer Cells: Bio macromolecules. 2008; 9 (10): 2905–2912
6. Kang Pan, Qixin Zhong, and Seung Joon Baek, Enhanced Dispersibility and Bioactivity of Curcumin by Encapsulation in Casein Nano capsules: Agric. Food Chem.2013; 61 (25): 6036–6043.
7. Uludag, De Vos P,Tresco P A. Technology of mammalian cell encapsulation: Adv. Drug Deliv. Rev.2004; 42, 29–64.
8. Antonietti, M. Self-organization of functional polymers: Nature Mater.2009; 2,9–10
9. Matyjaszewski K. Architecturally complex polymers with controlled heterogeneity: Science. 2011; 333:1104–1105.
10. Schenning A P H J, Benneker F B G, Geurts H M, Liu Y, Nolte R J M. Porphyrin wheels: J. Am. Chem. Soc.1996;118: 8549–8552.
11. Takazawa, K. Micrometer-sized rings self-assembled from thiocyanate dye molecules and their wave guiding properties: Chem. Mater.2007; 19: 5293–5301.
12. Carroll G T, Jongejan M G M, Pijper D, Feringa B L. Spontaneous generation and patterning of chiral polymeric surface toroids: Chem. Sci.2010; 1:469–472.
13. Kim, D. et al. Direct synthesis of polymer nanocapsules: self-assembly of polymer hollow spheres through irreversible covalent bond formation. J. Am.Chem. Soc. 2010;132: 9908–9919.
14. Kim D et al. Direct synthesis of polymer nanocapsules with a noncovalently tailorable surface: Angew. Chem. Int. Ed.2007; 46: 3471–3474.
15. Hota R et al. Self-assembled, covalently linked, hollow phthalocyanine nanospheres: Chem. Sci.2013; 4:339–344.
16. Nykypanchuk.D., Mayre, M.M., van der Lelie&Gang.O. DNA-guided crystallization of colloid nanoparticles: Nature,2008; 451:549-552(2008).
17. Park SYet al. DNA –programmable nanoparticle crystallization: Nature. 2008; 451:553-556.

18. Schappacher, M. & Deffieux, A. Synthesis of macrocyclic copolymer brushes and their self-assembly into supramolecular tubes: Science.2008; 319: 1512–1515.
19. Schappacher M, Deffieux A. Imaging of catenated, figure-of-eight, and trefoil knot polymer rings: Angew. Chem. Int. Ed.2009; 48: 5930–5933.
20. Bisht S, Mizuma M, Feldmann G, Ottenhof N A, Hong S M, Pamanik D, Chenna V, Karikari C, Sharma R, Goggins M G, Rudek M A, Ravi, Maitra A, Maitra, A. Systemic administration of polymeric nanoparticle-encapsulated curcumin (NanoCurc) blocks tumor growth and metastases in preclinical models of pancreatic cancer: Mol. Cancer Ther. 2010; 9: 2255–2264.
21. Bisht S, Feldmann G, Soni S, Ravi R ,Karikar C, Maitra, Maitra A. Polymeric nanoparticle-encapsulated curcumin (nanocurcumin): A novel strategy for human cancer therapy. J. Nanobiotechnol.2007; 5: 3–21.
22. Daniel S,Limson J L, Dairam A, Watkins G M, Daya S. Through metal binding, curcumin protects against lead- and cadmium-induced lipid peroxidation in rat brain omogenates and against lead-induced tissue damage in rat brain: J. Inorg. Biochem257. 2004; 98:266–275.
23. MaLing Gou, Ke Men, HuaShan Shi, MingLi Xiang, Juan Zhang, Jia Song, JianLin Long, Yang Wan, Feng Luo, Xia Zhao and ZhiYong Qian. Curcumin-loaded biodegradable polymeric micelles for colon cancer therapyin vitro and in vivo.1558 : Nanoscale. 2011; 3:1558–1567.
24. IM El-Sherbiny.“Enhanced pH-responsive carrier system based on alginate and chemically modified carboxymethyl chitosan for oral delivery of protein drugs preparation and invitro assessment,”: Carbohydrate Polymers.2010;80: 41125–1136. Australia
25. M El-Sherbiny, M. Abdel-Mogib, A.M. Dawidar,A. Elsaye, H D C Smyth. “Biodegradable pH-responsive alginate poly(lactic-co-glycolicacid) nano/micro hydrogel matricesfor oral delivery of silymarin,”: Carbohydrate Polymers.2011; ,83(3):1345–1354.
26. Tsutomu Hamada, Masamune Morita,Makiyo Miyakawa, Ryoko Sugimoto,Ai Hatanaka, Mun’delanji C Vestergaard, Masahiro Takagi. Size-Dependent Partitioning of Nano/Micro particles Mediated by Membrane Lateral Heterogeneity: J. Am. Chem. Soc.2012; 134 :13990−1399.
27. Kim D, Kim E, Kim J, Park K M, Baek K, Jung M et al .Direct synthesis of polymer nanocapsules with a noncovalentlytailorable surface.: Angew. Chem. Int. E2007; 46: 3471–3474 .