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Flow Through Diffusion Cell Method: A Better Approach to Study Drug Release Behavior as Compared to Traditional Dissolution Test Method

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Co-polymeric hydrogels consisting of N-vinyl-2-pyrrolidone (NVP) and acrylic acid (AAc) were synthesized and evaluated for release of a model drug, i.e., vitamin B_{12}. Release studies in simulated gastric fluid (pH 1.2) and intestinal fluid (pH 7.4), at 37°C, showed the hydrogels to be pH sensitive. An in vitro release study by 'traditional dissolution test' (TDT) showed that percent drug released from the hydrogel was nearly 8.6 ± 2.1 and 83.2 ± 4.8 in the media of pH 1.2 and 6.8, respectively. However, in order to incorporate in vivo GI conditions such as acidic pH and high water content in the stomach, low water content and the presence of a semi–solid mass in the large intestine, a new test model, called flow through diffusion cell (FTDC) was also used. The two approaches yielded almost different release profiles. The gels were characterized by thermogravimetric analysis and FTIR spectroscopy.

Keywords vitamin B_{12}, colon, traditional dissolution test (TDT), 'flow through diffusion cell' (FTDC)

Introduction

The last decade has witnessed tremendous research work in the field of colon–targeted drug delivery because the colon is considered as a suitable site for delivery of both conventional and labile drugs (1) and it is also a site for some special diseases such as ulcerative colitis, Chron's disease, bowel cancer, some infections and constipation which require local drug delivery (2). Various approaches have been used for colon–targeted drug delivery which include pH–dependent swelling controlled systems (3), delayed–release delivery systems (4), intestinal pressure–controlled colon delivery capsules (5) and enzymatically–degradable systems that utilize various enzymes produced by intestinal flora (6).

It has been well known that the traditional dissolution test (TDT), employed to evaluate the drug release behavior of an oral dosage form, involves measurement of release rate in the simulated gastric fluid (pH 1.2) and intestinal fluid (pH 6.8) under sink conditions. However, the experimental conditions maintained in TDT do not match

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with those of the human GI tract where the stomach has a high water content, while the large intestine has a low water content, low agitation intensity and dosage form is surrounded by undigested food particles. Therefore, it is required to improve the experimental conditions of the *in vitro* traditional dissolution test so that they almost match with the *in vivo* GI conditions and the possible behavior of a dosage form in the human GI tract can be predicted in more realistic way.

Recently, we have published results of *in vitro* release studies of vitamin B_{12} from poly(N-vinyl-2-pyrrolidone-co-acrylic acid) hydrogels (7) by employing a traditional dissolution test. In an attempt to incorporate *in vivo* GI conditions as mentioned above, we hereby report the results of release studies for the same drug/polymer system by a newly developed technique called 'flow through diffusion cell' (FTDC) method. The monomers used to synthesize gels have a fair reputation as non–toxic and biocompatible materials (8). Moreover, polyacrylic acid is known to be a good mucoadhesive and may increase the transit time of formulation (9). The model drug vitamin B_{12}, an organometallic compound with the cobalt atom situated within a corrin ring plays a key role in the reduction of disulfide groups and in maintaining substances like glutathione and coenzyme A in the reduced state, as well as the ratio NAD/NADH (10). The deficiency of vitamin B_{12} has caused diseases such as congenital pernicious anemia, gastrectomy, sprue, celiac syndrome, inflammation and ileal defects (11). There has also been some research on vitamin B_{12}–mediated transport of nanoparticles across Caco-2 cells (12).

Experimental

Chemicals

The monomers n-vinyl-2-pyrrolidone (NVP; Sigma, St. Louis, MO) and acrylic acid (AAc; Research Lab, Pune, India), the crosslinker N, N′–methylene bisacrylamide (MB; Research Lab), the initiator potassium persulphate (KPS; Merck, Mumbai, India) were of analytical grade. Acrylic acid was vacuum distilled at 47°C/7 mm Hg. Injections of vitamin B_{12} "Neurobion" (E. Merck, Mumbai, India, Batch Nos. 22308) were used as the model drug.

Synthesis of Hydrogels

The copolymeric hydrogels were synthesized by free radical polymerization of acrylic acid and n-vinyl-2-pyrrolidone in aqueous medium as described previously (7). The samples shall be denoted by HG(X) where X denotes the percent mole fraction of acrylic acid in the sample.

Drug Loading by Equilibration

The gels were loaded by equilibrating them in a drug solution of concentration 500 μg per ml for period of 24 h with constant stirring. The swollen gels were then air dried for 24 h, followed by vacuum drying for another 24 h at 40°C. The crystallized drug over the surface of the cylindrical gels was washed with distilled water and then vacuum-dried again. The drug–loaded samples shall be denoted by $HG(X)_y$ where 'y' represents the amount of drug in mg present in one gram of the polymer matrix. In this study, control sample is $HG(62)_{22.6.}$

Thermogravimetric Analysis and FTIR Spectral Analysis

The TGA was performed in the Indian Institute of Chemical Technology(IICT), Hyderabad, India, using a thermogravimetric analyzer(Mettler, Teledo TGA/PVP 9959) controlled by STAR software(Mettler Toledo GmbH, Switzerland). About 4.6 mg of a powdered hydrogels sample is $HG(62)_{22.6.}$ placed in ceramic crucible and analyzed over the temperature range at 32°C to 1000°C at the rate 10°C min^{-1} under the dry flow of N2 at the rate of 30 ml/min.

The FTIR spectrum of the hydrogel sample $HG(62)_{22.6.}$ was recorded using a Perkin–Elmer 1600 Fourier Transform IR spectrophotometer with a resolution of 4 cm^{-1} and averaged over 32 scans. The dry hydrogel sample was crushed with potassium bromide and pellets were formed under a hydraulic pressure of 600 kg/cm^2.

Drug Release Studies

For the purpose of carrying out *in vitro* drug release studies by a traditional dissolution test, buffer solutions of required pH were prepared (HCl for pH 1.2 and phosphate buffer for pH 6.8) and three pre-weighed samples were put each in 25 ml buffer solution at the physiological temperature 37°C with a constant agitation speed of 50 rpm. The amount of drug released at different time intervals was determined spectrophotometrically at 375 nm (13). After each observation, gels were put in a fresh buffer solution. The amount of drug released was computed by comparing the absorbance with the standard curve prepared for the pure drug in the appropriate concentration regions.

As mentioned earlier, the release kinetics was also studied by a newly developed approach called the FTDC method, which has been depicted in Figure 1. A cylindrical cell with a cone–shaped bottom (in accordance with the dissolution test method 3 of JP) was filled with swollen acrylamide hydrogel particles (initially sieved to 600 μm) as a filler. The test medium (HCl of pH 1.2 as JP 1st fluid and phosphate buffer of pH 6.8 as JP 2nd fluid) is dropped from the upper side of the cell at a constant flow rate and the outflow from the bottom of the cell is collected in fractions at different time–intervals. Since the dropped medium drains through the swollen gel particles into the bottom and the volume of the medium in the cell remains consistently small, this mimics the presence of undigested food particles and the low water content conditions of the large intestine.

Results and Discussion

Thermogravimetric Analysis

The results of thermogravimetric analysis have been tabulated in Table 1. It is clear from the thermogram (see Figure 2) that the hydrogel possesses sufficient stability and may be used at the physiological temperature.

FTIR Spectral Analysis

The FTIR spectra of poly(NVP–co–AAc), as depicted in Figure 3 indicates the broad band appeared at 3220–3600 cm^{-1} (due to H–bonded hydroxyls), methylene group at 2925 cm^{-1} (due to asymmetric stretching i.e., ν_{as} CH_2) and at 2786 cm^{-1} (symmetric stretching i.e., ν_s CH_2) carboxylic groups at 1759 cm^{-1} (C═O stretching band), 1642 cm^{-1}(due to asymmetrical stretching of —COO^-) and 1464 cm^{-1} (due to symmetrical stretching of —COO^-).

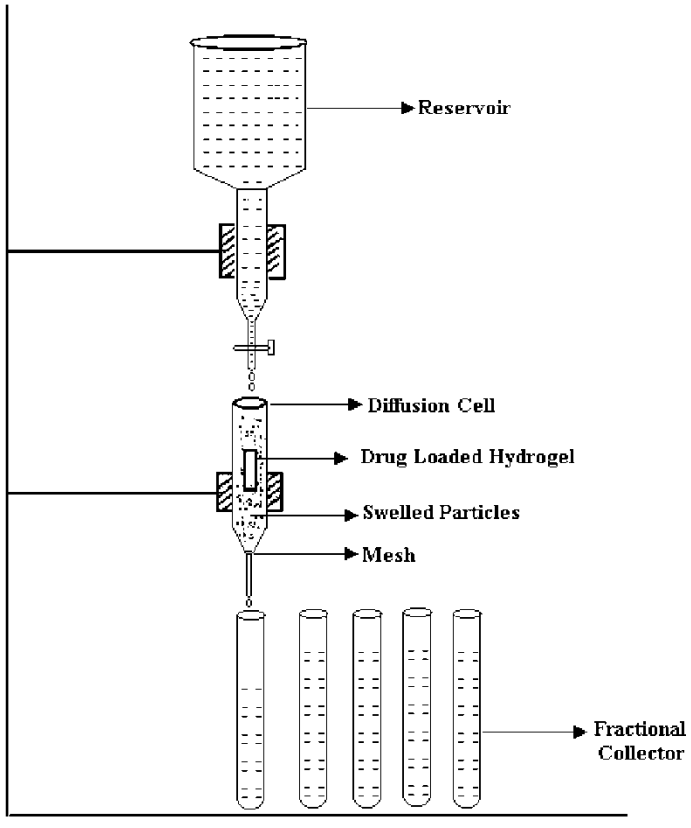

Figure 1. Schematic diagram of apparatus employed for 'flow through diffusion cell' method.

The prominent band observed at 2170 cm^{-1} may be attributed to the presence of —C—N group of crosslinking agent, i.e., N,N′–methylene bisacrylamide.

Due to absorption from vitamin B_{12}, the bands seems to be merged with the broad peaks of the polymer. However, on comparison of bands due to νNH and amide I (involving main contribution from νC═O) it can be inferred that vitamin B_{12} has been observed into the polymer by hydrogen bonding interactions. Finally, it can be concluded that there is physical entertapment of vitamin B_{12} in the polymer matrix. However, the main features of the IR spectra of polymer remains unaffected thus signifying purely physical type of entrapment of drug in the polymer matrix.

Table 1
Data for thermogravimetric analysis of the sample HG(62)

Sample code	HG(62)
T_{id} (°C)	150
T_{fd} (°C)	750
T_{max} (°C)	250
% Wt at 133°C	99.85

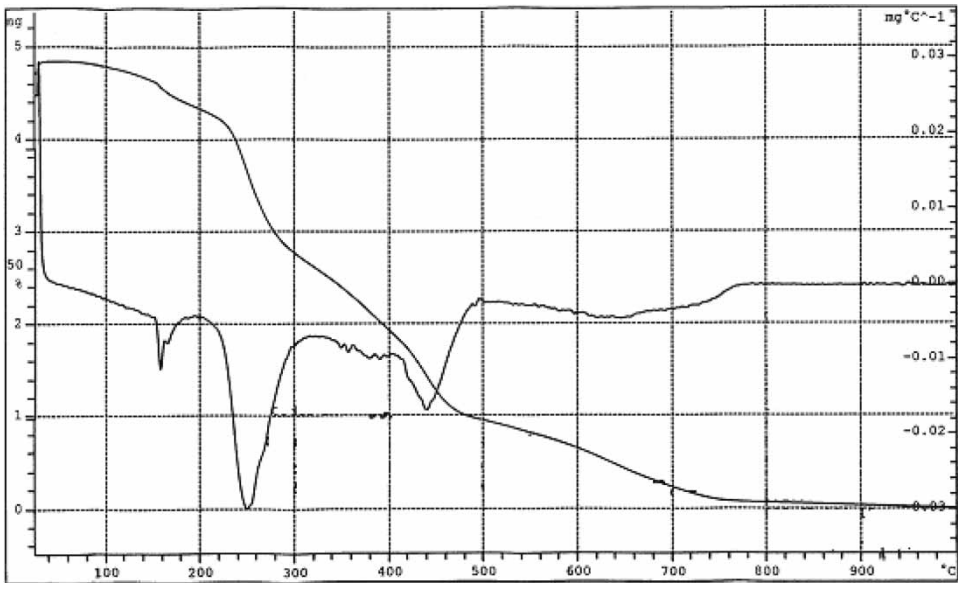

Figure 2. Thermogram of the hydrogel sample $HG(62)_{22.6}$.

Dynamic Release at Different pH

As the proposed hydrogel system shows a fair pH–dependent swelling (7), it may bear potential to be used for colon–targeted drug delivery as it is expected to demonstrate minimum release in the acidic pH and maximum in the medium of pH 6.8. To confirm this, dynamic release of sample $HG(62)_{22}$ was studied in the media of pH 1.2 and 6.8 at 37°C. Results, as depicted in Figure 4, clearly indicate that the hydrogel

Figure 3. IR spectra of (a) Plain hydrogel sample; (b) Model drug vitamin B_{12} loaded hydrogel sample.

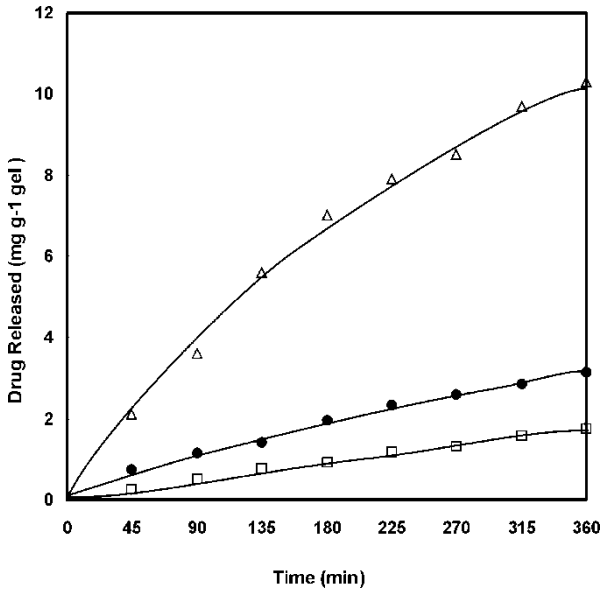

Figure 4. Dynamic release of vitamin B_{12} from the hydrogel sample $HG(62)_{22.6}$ as a function of time in the medium of pH 1.2 (□), 4.0 (△) and 6.8 (●) at the physiological temperature 37°C using traditional dissolution test (TDT).

releases amaximum amount of B_{12} in the medium of pH 6.8, while minimum drug is released in the medium of pH 1.2. The percentage of the total drug released from the device $HG(62)_{22}$ in the release media of pH 1.2 and 6.8 was found to be 8.63 ± 2.1 and 83.2 ± 5.8, respectively. The observed findings may be explained on the basis of the fact that macromolecular chains of the polymer network undergo extensive chain relaxation in the medium of pH 6.8 due to electrostatic repulsion among the similarly charged —COO^- groups produced due to ionization of —COOH groups, This finally results, in extensive swelling with subsequent drug release. However, in the medium pH 1.2, the unionized —COOH groups form a H–bonded compact gel structure and restricts the movement of polymeric segments within the gel, thus causing minimum release.

Evaluation of Diffusion Coefficient

For cylindrical hydrogels, the integral diffusion at short times, as derived from Ficks first and second law of diffusion, is given as:

$$F = 4\left[\frac{(Dt/l^2)^{1/2}}{\pi^{1/2}}\right] \tag{1}$$

Here, F is the fractional release (M_t/M_∞); M_t and M_∞ are drug released at time 't' and at equilibrium, respectively, D is the diffusion coefficient and l is radius of the sample. In Equation (1), the slope of the linear plot between F and $t^{1/2}$ yields D. However, it is not unusual to observe that F is not linear with $t^{1/2}$ (14). This non-Fickian behavior is often found for diffusion into glassy polymers below their T_g. Therefore, initial diffusion coefficient D_i was evaluated from the initial linear portion of the plot.

The average diffusion coefficient D_{av} is also calculated for 50% of the total release by putting $M_t/M_\infty = 0.5$ in the Equation (1), which finally gives:

$$D_{av} = \frac{0.049\ l^2}{t^{1/2}} \tag{2}$$

where $t_{1/2}$ is the time required for 50% release of the drug from the device.

Diffusion coefficient were also calculated using the late–time approximation as described by N.A. Peppas (15).

$$\frac{M_t}{M_\infty} = 1 - \left[8/\pi^2 \left\{\exp\left(\frac{-\pi^2 D_L t}{4l^2}\right)\right\}\right] \tag{3}$$

A plot between ln $(1 - M_t/M_\infty)$ and t was used to evaluate D_L.

Flow–Through Diffusion Cell (FTDC) Methods

The conditions of *in vitro* drug release studies by the traditional dissolution test (TDT) do not reflect the *in vivo* GI conditions. So, the release studies were also performed using the newly developed technique 'flow through diffusion cell' (FTDC) method (16).

Figure 5 displays a comparative depiction of release profiles demonstrated by the formulation $HG(62)_{22}$ in the medium of pH 6.8 as studied by traditional dissolution test (TDT) and flow through diffusion cell (FTDC) method with swollen gel particles (cross-linking ratio 0.023, particle size 600 μm) used as fillers. It is very clear that the amount of drug released at various time–intervals is more in the FTDC method, while a slower release is observed in the TDT. One may expect a faster release of drug in TDT as the

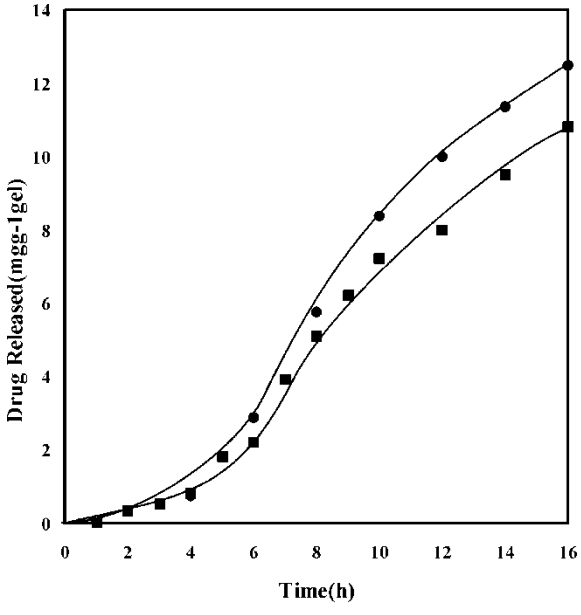

Figure 5. Comparison of *in vitro* drug release profiles of formulation $HG(62)_{22.6}$ in medium of pH 6.8 obtained by TDT (■) and FTDC with swollen polyacrlamide gel particles used as fillers (●) at the physiological temperature 37°C.

drug–loaded device sinks in the release medium. However, the hydrogel demonstrated faster release in FTDC which may be explained as follows:

The diffusion of drug through a swelling dependent device depends upon a number of factors like concentration gradient developed within the device, mesh size of the polymer network, and partition coefficient of the drug between the gel phase and release media, etc. However, the concentration gradient developed at the gel solution interface seems to be a governing factor in the present situation. In TDT, the dosage form was put in the 25 ml of buffer solution and the release medium was replaced after definite time–intervals (which was 2 h in the present case) by a fresh buffer solution. It means that for a period of 2 h, the drug released from the device remains in the surrounding fluid and hence, there are chances of its sorption (although to a very small extent) back into the swelling device. Moreover, the presence of drug in the vicinity of the device may also result in lowering the concentration gradient at the gel-solution interface. This finally results in a slower release from the gel. On the other hand, in the FTDC method, the drug released from the device goes down along with the release medium, which is dripping down at a constant flow rate. In this way, as soon as the drug comes out of the polymer network, it is removed from vicinity of the gel along with the medium. Therefore, the chances of sorption of drug back into the swelling network are almost nil. Moreover, the immediate removal of the released drug may develop a sharp concentration gradient at the gel–solution interface. Thus, a faster–diffusion is observed in the FTDC method. Various diffusion coefficients, calculated for the diffusion of drug from the polymer matrix, as studied by TDT and FTDC methods have been given in the Table 2.

Effect of Nature of Filler Particles on Release Profiles

The nature of semi–solid, surrounding the dosage form may influence the drug releasing capacity of the device. To investigates this, the dynamic release of B_{12} was studied by the FTDC method with swollen gel particles and glass beads (diameter 0.3 cm) used as fillers. The results, as depicted in Figure 6, clearly suggest that the presence of glass beads results in faster release as compared to the swollen gel particles. This may be attributed to the fact that the hydrophobic surface of the glass beads possesses water-repelling tendency, and therefore, the release medium has greater opportunity to diffuse into the gel with subsequent release of drug. In addition to this, as the diameter of the beads is sufficiently large as compared to that of swollen gel particles (0.3 mm vs. 600 μm), the glass beads

Table 2
Various diffusion coefficients evaluated for the release of vitamin B_{12} from the sample HG $(62)_{22.6}$ by TDT and FTDC method at the physiological temperature 37°C

	Diffusion coefficients		
Method	Initial $D_i \times 10^6$ ($cm^2\,min^{-1}$)	Average $D_{av} \times 10^6$ ($cm^2\,min^{-1}$)	Late time $D_L \times 10^6$ ($cm^2\,min^{-1}$)
TDT	5.68	58.39	31.74
FTDC	0.065	9.2	600

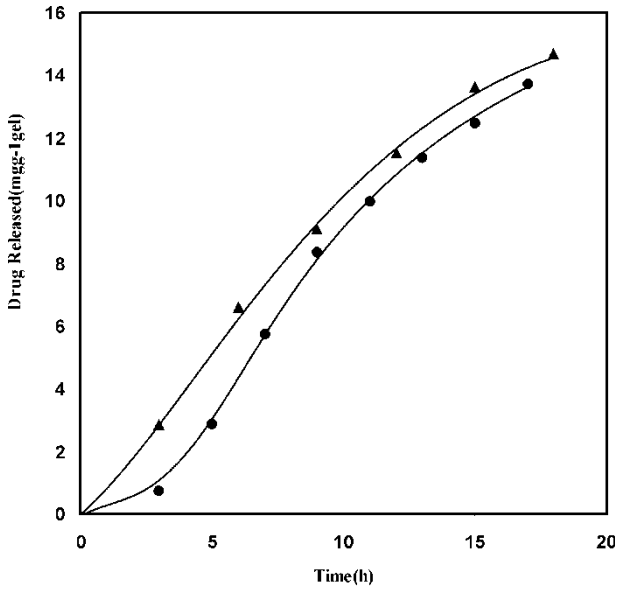

Figure 6. Comparison of *in vitro* drug release profiles of the drug–loaded sample $HG(62)_{22.6}$ in the medium of pH 6.8 obtained by FTDC methods using swollen gel particles (●) and glass beads (▲) as fillers.

occupy less volume in the space available in the diffusion cell, thus forming fixed wider water channels through which drug diffuses at faster rate. On the other hand, swollen gel particles occupy more space surrounding the hydrogel sample and may form discontinuous and narrow water channels through which diffusion of solvent towards the device becomes comparatively slow. Finally, the average diffusion coefficients for the permeation of drug in the presence of glass–beads and swollen gel particles were found to be 11.9×10^{-6} and $9.2 \times 10^{-6} \text{cm}^2\,\text{min}^{-1}$, respectively.

Therefore, we see that the *in vitro* method followed to study the release profiles and the environment created surrounding the drug–loaded device affect the release behavior of the device.

Conclusions

The release of model drug vitamin B_{12} has been studied by the traditional dissolution test, as well as by the newly developed 'flow–through diffusion cell' method. The results obtained clearly indicate that the two approaches yield different drug release profiles.

It has been found that drug is released at a slower rate in TDT, whereas the FTDC method displays faster release. Moreover, in the FTDC method, the device has been observed to exhibit faster release when surrounded with glass beads rather then with swollen gel particles. Therefore, it can be concluded from this study that a dosage form, intended to be employed for the oral delivery along GI tract, should be studied by a flow through diffusion cell method for its drug release behavior, as this method involves such conditions, which to some extent, reflect the *in vivo* GI conditions. So, this method is better than the TDT, which is carried out under sink conditions only. Lastly, it is noteworthy to mention here that the method can be tested by

comparing *in vivo* release data of a polymer/drug system with that obtained using the FTDC approach and thus obtaining the IVIVC (*in vitro-in vivo* correlation) using the similarity factor (17).

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