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Ester Prodrug of 5-Aminosalicylic Acid for Colon Specific Drug Delivery: Synthesis, Kinetics, Hydrolysis and Stabilities studies.

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Abstract
Acrylic type polymeric systems having degradable ester bonds linked to the 5-aminosalicylic acid (5-ASA), were synthesized and evaluated for colon targeted drug delivery. The obtained prodrug was characterized by FTIR,^{1}HNMR,Melting point and R_f value.In vitro drug release study was conducted at pH 1.2,7.4 and in rat fecal matter. Drug release in rat fecal matter at pH 7.4 was found to be most satisfactory. A burst release of 41.65% was observed in the first two hours followed by a sustained release over a period of 12 hours. A maximum of 92.46% of the drug was released from the prodrug and the time taken for 50% drug release (t_{50}) was found to be nearly 3 hours. The best linearity for the prodrug was found in Higuchi's equation, where r^2 value was 0.9852, indicating the release of drug from prodrug as square root of time dependent process based on Fickian diffusion.The result suggest that these systems could be useful for preparation of a controlled release formulation of 5-aminosalicylic acid to target colon.

Key words:-5-aminosalicylic acid,2-hydroxy ethyl methacrylate,IBD,polymeric prodrug.

1. Introduction

Inflammatory bowel disease (IBD) is characterized by chronic inflammation in the mucosal membrane of the large intestine. Although many treatments have been recommended for IBD, they do not treat the cause but are effective only in reducing the inflammation and accompanying symptoms in up to 80% of patients. The primary goal of drug therapy is to reduce inflammation in the colon that requires frequent intake of anti-inflammatory drugs at higher doses. 5-Aminosalicylic acid (5-ASA) is very effective in IBD but it is absorbed so quickly in the upper gastrointestinal tract (GIT) that it usually fails to reach the colon leading to significant adverse effects. [1]

To overcome these problems various approaches such as liquid formulations, parentral and rectal administration, enteric coated tablets, pro-drugs and microparticulate systems have been made. Further, several sustained release formulations based on polymer matrix or reservoir systems have also been used. Co therapies with sucralfate, H_2 antagonists, proton pump inhibitors, prostaglandin inhibitors were also used. All these, however, were not able to provide complete protection against the GI complications.

A more novel approach is the preparation of polymeric drug derivatives, where the drug molecules are covalently linked to the polymeric backbone. Linkages with limited stability in the physiological environment can be used. This approach should modify the pharmacokinetics of the drug and also obtain preferential localisation.

If a polymeric pro-drug wherein, the drug is covalently attached to the polymeric backbone is synthesised, and if it is capable of cleaving itself and release the drug in the alkaline environment (lower GIT) rather than the acidic environment (upper GIT), this should avoid direct contact with the gastroduodenal mucosa and thus prevent local imitation. Such a system would also prolong the pharmacological response of the drug thus leading to a good sustained release system. Dose dumping, associated with other conventional reservoir systems, can also be avoided here.

The objective of the present work is, therefore, to synthesise and evaluate polymeric pro-drug containing a non-steroidal anti-inflammatory drug, namely 5-Aminosalicylic acid, for sustained and site- specific delivery and evaluate their in vitro release behaviour.

For the synthesis of the polymeric pro-drug a polymeric drug carriers, namely, poly (hydroxyethyl methacrylate); [poly (HEMA)]

was chosen because this carrier has a poor tendency to absorb biological species as a result of which they show good biocompatibility.They also have low interfacial energies in aqueous solutions. Moreover, they are also expected to be excreted as such since they are not absorbed by mucosal surfaces [2]

Among the several linkages that were proposed, the ester linkage was proposed to be used because it is perhaps the most appropriate covalent linkage for attaching the anti-inflammatory drugs (5-ASA) to the polymeric carriers. This is because, the ester linkage not only shows relatively stability in the acidic environment but also hydrolyses easily in physiological basic medium. Thus, the amount of drug released is more in the lower GI tract. Although hydrolysis of the pro-drug in the upper GI tract takes place, it is relatively much less since the residence time of the drugs in the upper GI tract is about two hours whereas, in the lower GI tract it is much higher. [3]

The pro-drug was proposed to be synthesised by initially obtaining the monomeric drug derivatives, characterising them and then polymerising them by suitable polymerisation techniques. This procedure was considered suitable for the preparation of the pro-drugs since it would result in polymeric pro-drugs with 100% degree of substitution which is required for higher yields of drug release. [4]

5-Aminosalicylic acid was proposed to be studied in the synthesis of the pro-drug because it is weak carboxylic acid with a pKa value of 3-4 range. In the gastric pH this is present as an unionised molecule. It is known that cell membranes are more permeable to unionised molecules than the ionised ones because of greater lipid solubility. The drug is, therefore, absorbed predominantly in the upper GI tract. Local irritation to the mucosal surfaces is more likely. The pro-drug was proposed to be synthesised with the aim that it would convey the release of the drug in the lower GI tract, in a site-specific manner thus avoiding local side effects.[5]

The pro-drug has been evaluated for their in vitro drug release behaviour at pH 1.2 ,7.4 and in rat fecal matter at pH 7.4, stimulating the upper and lower GI tract to assess their capability to release the drug largely in the alkaline environment of the lower GI tract. Prodrug was fitted to various models such as zero-order, first-order, Higuchi, Hixcon-crowel, Korsmeyer and peppas to ascertain the kinetic modeling of drug release. Hydrolysis & stability studies of the prodrug were also conducted to analyze the same.

MATRIALS & METHODS :

5-Aminosaliculic Acid was purchased from Acros Organics (Thermo Fisher Scientifics), 2-Hydroxy ethyl metha acrylate (HEMA) was obtained from Sigma Aldrich Life Sciences, New Delhi, Thionyl chloride was obtained from S.D fine chemicals, DMSO was purchased from Ranbaxy fine chemicals, Benzoyl Peroxide from Spectrochem Pvt. Ltd, Mumbai; all other chemicals were reagent grade or purer.

Preparation and Characterisation of 5-Aminosalicylic pr odrug:- 2-Hydroxyethyl methacrylate (HEMA) [12.2 ml] was taken in a 250ml double necked round bottom flask fitted with a stirrer and condenser. The flask containing HEMA was then heated in an oil bath to 40°C. Thionyl chloride (7.2 ml] was added dropwise to the reaction flask using a dropping funnel. When the addition was complete, the temperature of the flask was raised to 70^0C and the flask was stirred at this temperature for 3-4 hours. The excess of thionyl chloride was removed by distillation and the chloro ethyl methacrylate derivative obtained as a liquid was distilled and used for further experiments. FTIR spectrum was done to analyze the chloro derivative of HEMA.

The monomeric drug derivative was prepared by reacting the sodium salt of 5-Aminosalicylic Acid with Chloroethyl methacrylate. The 5-ASA (5.25gm) was taken in a double necked round bottom flask fitted with a stirrer and condenser, now Dimethyl sulphoxide (50ml.) was added to the flask and stirred until the drug was completely dissolved. Chloroethyl methacrylate (4.17ml) was then added and the flask was heated to 120^0C and maintained at this temperature with constant stirring for 10

hours. After the reaction period the contents in the flask were poured into 500ml. of distilled water with vigorous stirring. A light brown precipitate was obtained which was allowed to settle overnight. The precipitate was filtered and dried. The monomeric drug derivative was purified by dissolving in 20ml acetone and reprecipitating it by pouring into 200ml distilled water. It was purified by crystallisation. The monomeric drug derivatives were characterised by FTIR spectra, thin layer chromatography, physical appearance and melting point.

The monomeric dug derivative of hydroxyethyl methacrylate (HEMA) [5gm.] was taken in a double necked round bottom flask fitted with a stirrer and condenser. Dimethyl sulphoxide (50 ml.) was added and stirred to dissolve the monomeric derivative. Nitrogen gas was allowed to bubble through the reaction mixture throughout the reaction period. The flask was heated in a water bath to 70^0 C. Benzoyl peroxide (0.1 g.) was then added to initiate polymerisation. The reaction was stirred for 8 hours. After the reaction period, the flask was cooled to room temperature and the contents were poured into 500 ml. of distilled water. A light brown precipitate was obtained. The precipitate was filtered and dried. The polymeric pro-drug thus obtained was purified by dissolving it in 20 ml. of acetone, reprecipitating it from distilled water and drying at reduced pressure to constant weight. The absence of HEMA was confirmed by a single spot in thin layer chromatography. Polymeric prodrug was confirmed by ^{1}HNMR spectra, thin layer chromatography, physical appearance and melting point.

Estimation of drug content:- Polymeric pro-drugs (100 mg.) synthesized was dissolved separately in 100 ml of 0.1 M. sodium hydroxide solution containing 2% w/v rat fecal material which were then kept overnight for complete release of the drug from its pro-drugs by hydrolysis. From this 5 ml each were transferred separately into a 100 ml. volumetric flask and diluted upto the mark with acetonitrile and the absorbance was measured at their respective λmax (300nm). [6]

In vitro drug release study:-In vitro drug release study was carried out by placing 100 mg prodrug containing a known amount of drug into a hard gelatine capsule. The study was carried out in different pH levels i.e. pH 1.2, and pH 7.4. Samples of 5 ml. were withdrawn at time intervals of 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours. The absorbances of the samples withdrawn after suitable dilution were measured against the reagent blank at their respective λmax (300nm for pH 1.2 and 338 for pH 7.4) of the drug determined.[7]

Release study in rat fecal matter (pH 7.4):- The prodrug was dissolved separately in phosphate buffer (pH7.4) so that final concentration of the solution was 250µg/ml. Fresh fecal material of rats was weighed (about 1g) and placed in different sets of test tubes. To each test tube, 1ml of the prodrug solution was added, and diluted to 5ml with phosphate buffer (50 µg/ml). The test tubes were incubated at 37ºc, for different time intervals (0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours). For analysis, the concentration of released drug from prodrug was estimated on a double beam UV Spectrophotometer at 338nm of λmax through their calibration curve. [8]

Kinetic modelling of drug release: The dissolution profile of the prodrug was fitted to various models such as zero-order, first-order, Higuchi, Hixcon-crowel, Korsmeyer and peppas to ascertain the kinetic modeling of drug release. [9]

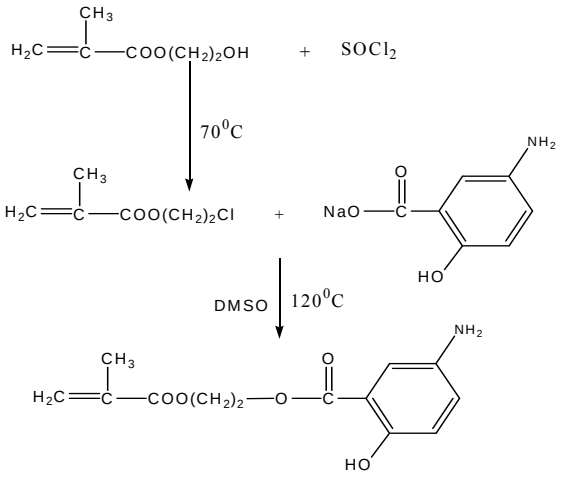
Characterization of hydrolysis product :- Twenty milligram of the polymer-drug conjugates was dispersed into 20 ml of buffered solution (pH 7.4) and maintained at 37 °C. After 24 h, the hydrolysis solution was sampled, neutralized with 1 N HCl and the solvent was removed in vacuum. The resulting crude product was treated with 10 ml of acetone and heated. The suspension was then filtered and the acetone solution was

evaporated under reduced pressure. The residue was characterized by melting point measurement, R^f value (TLC method) and FTIR spectroscopy [10]

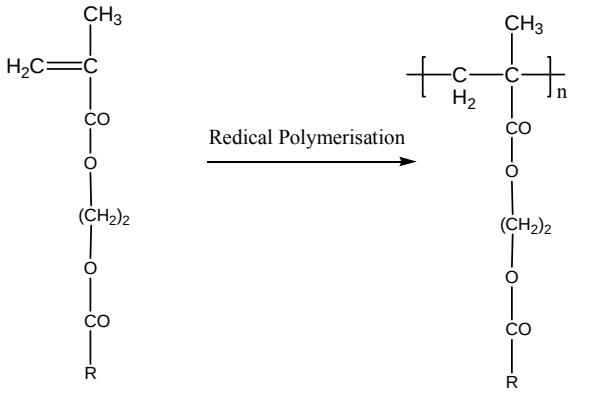
Stability studies: - The stability of the prodrug at room temperature (20°C-25 °C) was carried out over a period of 3 months. Sample was withdrawn at the end of 30, 60, and 90 days and analyzed for physical appearance, melting point, FTIR spectra and drug content while in vitro drug release study was carried out directly after 90 days to analyze the f_1 (difference factor) & f_2 (similarity factor) factors in release pattern.[11]

RESULTS AND DISCUSSION

The synthesis scheme for the polymeric prodrug is given in Fig.[1]. The FTIR spectrum of the chloro derivative of HEMA) shows the absence of any signals in the region of 3000 to 3500 cm^{-1} and the presence of characteristic bands at 1722 cm^{-1} (C=O), 1636 cm^{-1} (C=C) and 759 cm^{-1} (C-Cl), thus indicating the replacement of the hydroxyl group by chlorine. The monomeric drug derivative has a dark brown color with melting point 310^{0}C (while m.p of 5-ASA is 283^{0}C) and R_f value 0.72 (while R_f value of 5-ASA is 0.43), indicating the formation of new product. Prodrug which is synthesized from 5ASA and HEMA retained all the peaks for 5-ASA like 3298.05 cm^{-1} (NH stretching), 1685.67 cm^{-1}(C=O stretching), 3358 cm^{-1}(OH stretching), 833.19 cm^{-1}(C-H out of plane bending) with some extra peaks like at 1558 cm^{-1} for C=C stretching, 2918.10 cm^{-1} for C-H aliphatic stretching. A less intensive peak was found at 1701 cm^{-1} indicating the ester bond presence in synthesized prodrug.



Step1- Monomeric drug derivative



Step 2- Polymerisation of monomeric drug derivative

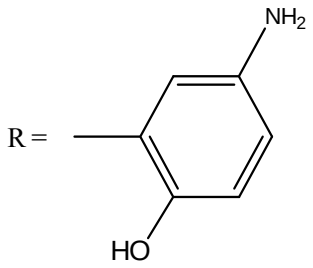


Fig.1 Synthesis of the polymeric prodrug

Polymerisation of the monomeric drug derivative was charcterized by a light brown color drug derivative with melting point 322^0C (while m.p of monomeric drug derivative and 5-ASA are 310^0C and 283^0C respectively) and R_f value 0.67 (while R_f values of monomeric drug derivative and 5-ASA are 0.72 and 0.43 respectively), indicating the formation of new product. ^{1}HNMR d DMSOd6, 300 MHz) δ 1.912 (S, 3H, $=\overset{CH_3}{\overset{|}{C}}-$), 2.313 (S, 2H, - CH_2=), 4.256 (t, 2H, OCH_2), 4.365(t, 2H, $COOCH_2$), 7.475 (m, 3H, ArH), 7.957 (S, 1H.Ar-OH). Fig.[2]

Estimation of drug content from the prodrug was done and each of 100 mg prodrug was contained 53.56 of drug content. While in vitro release profile is given in Fig.[3].The polymeric produg shows sustained & targeted drug release behaviour over a period of 12 hrs where a maximum of 16.95% of the drug was released over a period of 12 hours while the drug release in the first two hours was only 5.19%. Drug release at pH 7.4 was found to be significantly more compared to pH 1.2. A maximum of 30.63% of the drug was released over a period of 12 hours while the drug release in the first two hours was only 11.64%. Drug release in rat fecal matter at pH 7.4 was found to be most satisfactory.

A burst release of 41.65% was observed in the first two hours followed by a sustained release over a period of 12 hours. A maximum of 92.46% of the drug was released from the prodrug and the time taken for 50% drug release (t_{50}) was found to be nearly 3 hours.

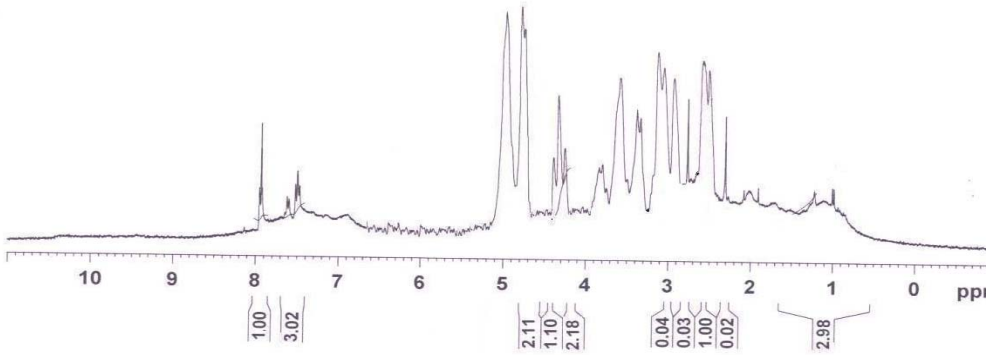


Fig.2 ^{1}HNMR spectra the polymeric drug derivative

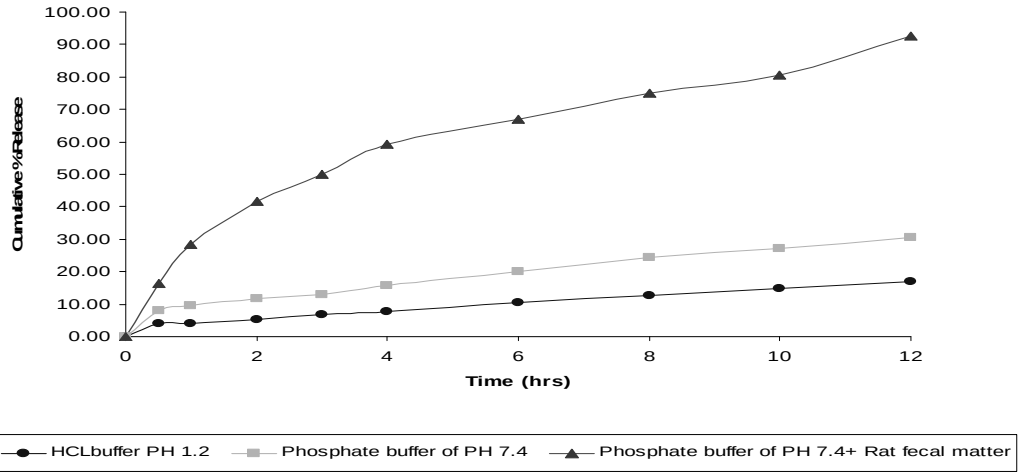


Fig.3:- Graph showing a comparison of in vitro drug release profile of polymeric prodrug at pH 1.2, pH7.4 and in rat fecal matter

In order to explain the in vitro drug release data and the sustained and site-specific nature of drug release envisaged in the present study, an understanding of the drug release mechanism is essential. Drug release in the case of the polymeric pro-drug synthesised should depend on the nature of the functional group undergoing hydrolysis and steric hinderence. There are two hydrolysable ester groups, one adjacent to the polymeric backbone and the other relegated to the pendant chain by a spacer group. It is obvious that hydrolysis of the latter ester group is much more facile than the one adjacent to the polymeric backbone because of steric reasons.

In the alkaline environment of the lower GI tract, however, hydrolysis is mainly take place by microfloral enzymes. Where esterase enzyme released by microbes is expected to hydrolyse the ester linkage and releasing the free drug. In other words base hydrolysis reactions proceed to completion Even allowing for a certain amount of hydrolysis of the pro-drug in the acidic environment of the upper GI tract, the amount of drug released here should be much less because the residence time in the upper GI tract (stomach and duodenum) is less than two hours .As well as a certain amount of hydrolysis of the pro-drug taken place even in absence of microfloral enzymes due to a nucleophilic attack of the hydroxyl group on the electron deficient carbonyl carbon in the lower GI tract, but the most of drug release takes place predominantly in the lower GI tract only in presence of microfloral enzymes especially esterase which is expected to hydrolyse the ester linkage and releasing the free drug thus allowing for site-specific delivery .

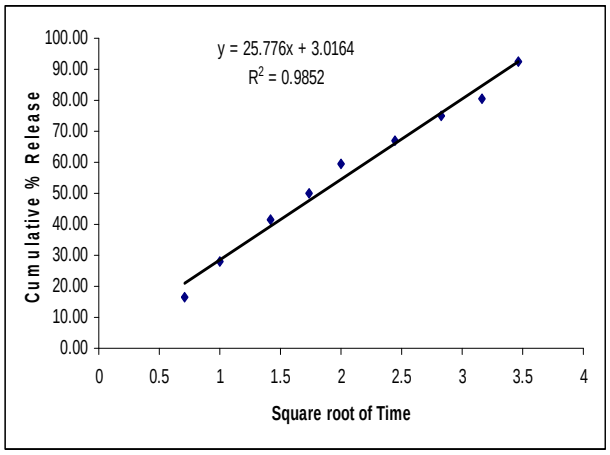


Fig.4:- Higuchi release model of polymeric prodrug of 5-ASA

Kinetic modelling of drug release was fitted to various models .The best linearity for the prodrug was found in Higuchi's equation plot Fig. [4] where r^2 value was 0.9852 that is close to one, indicating the release of drug from prodrug as square root of time dependent process based on Fickian diffusion.

After hydrolysis, the residue was characterized & confirmed as 5-ASA Fig. [5] because its melting point and R^f value was found 283^0C and 0.43 respectively. While FTIR spectra shows 3388.70^{cm-1}(OH stretching), 3298.05cm^{-1}(NH stretching), and 3066.61 $^{cm-1}$ (CH aromatic stretching), 1685.67 $^{cm-1}$ (C=O stretching), 833.19cm^{-}1(CH out of plane bending) .These all are the characteristics of 5-ASA.

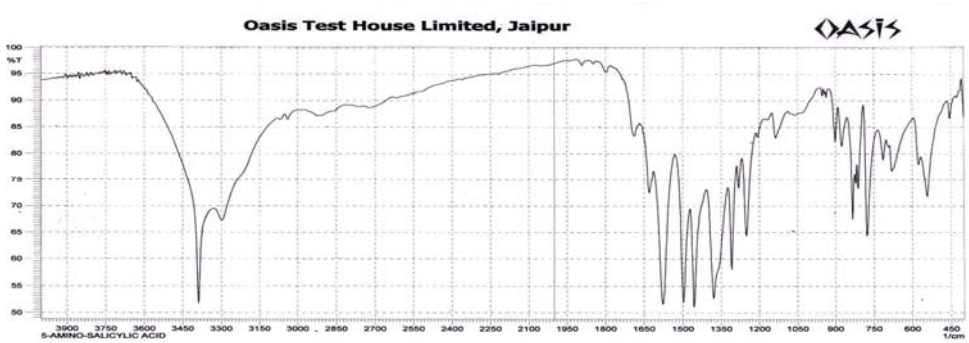


Fig.5:-FTIR Spectrum of 5-ASA (Product after hydrolysis of Prodrug)

Stability studies showed no significant change in the physical appearance, melting point, and its FTIR spectra. While in vitro drug release behaviour when compared by mean of similarity & difference factors ,the result indicated that their similarity factor was found to be more than 98 that was in the range between 50-100 (according to FDA), ensuring the sameness of products. While the difference factor was found to be less than .2 that was in the range between 0-15 (according to FDA), ensuring minor difference between two products. Therefore there is no significant change in the release pattern, indicating no changes occurred during storage.

CONCLUSION

In this work, HEMA polymeric prodrug containing 5-ASA was synthesized. The structure of the obtained prodrug was characterized ^{1}HNMR, FTIR, Melting point and R^{f} value. The release profiles of 5-ASA from prodrug showed that the drug release in rat fecal matter at pH 7.4 was found to be most satisfactory. A burst release of 41.65% was observed in the first two hours followed by a sustained release over a period of 12 hours. A maximum of 92.46% of the drug was released from the prodrug and the time taken for 50% drug release (t_{50}) was found to be nearly 3 hours. However, a certain amount of 5-ASA can be released by hydrolysis of the polymeric produg in small intestine (pH 5–7), but the amount of released 5-ASA in colon (pH 7.4) is very high. Therefore, the studied polymers in the present investigation can be used in the achievement of controlled drug release or slow release, prolongation of transit time and are useful as drug carriers for development of colon targeted delivery.

The prodrug therefore is expected to reduce the frequency of administration and avoid the gastrointestinal adverse effects associated with 5-ASA as a drug.

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