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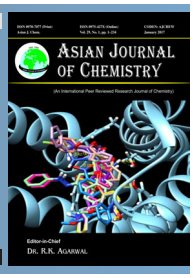

Critical Modification to Bulk Scale Synthesis of 2-Amino-5-carboethoxy-4-hydroxypyrimidine

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Pyrimidines and pyridines are choice for modern day medicinal chemistry. Pyrimidine synthons are synthesized form guanidine and different substituted malonates. One of such pyrimidine ring synthon is 2-amino-5-carboethoxy-4-hydroxy pyrimidine. The commercially deployed synthesis involved a mixture of KOH and guanidine carbonate and gradual temperature controlled addition of diethylethoxy methylene melonate giving a yellow precipitate. The precipitate is cooled to around 5 °C and recrystallized from ethanol-water mixture. Though purity is never an issue in this popular process, yields are very low (70-75 %). The GC analysis of reaction mixture indicated that almost starting material was left unreacted and the first yellow precipitate formation is the rate determining step. We report silica functionalized magnetic particles as material support for synthesis of 2-amino-5carboethoxy-4-hydroxy pyrimidine. The cyclization reaction yields are reported to be enhanced due to presence of "near-homogeneous" nanomaterial catalyst. The prominent catalyst of interest to us is Fe_3O_4@SiO_2 of 40 nm size. The particles are produced by modified Stober process giving consistent yields and coating of SiO_2. The structural characterization is performed with SEM, TEM, IR and the data are consistent across multiple batches. The use of 40 nm size Fe_3O_4@SiO_2 enabled higher yields of cyclization step in synthesis of 2-amino-5-carboethoxy-4-hydroxy pyrimidine. The mechanism of catalysis is stabilization of hetero atoms on the acidic silica surface and hence formation of pyrimidine. The study with different sized nanoparticles has indicated 40 nm size seems to be optimum and ability of catalysis is reduced as the size of nanoparticles has increased. The reaction performed at different batch sizes has indicated that 5 % (w/v) catalyst is optimal in the reaction. This process modification has far reaching applications in medicinal chemistry and bulk drug synthesis.

Keywords: Pyrimidine, Magnetite nanoparticles, Solid support, 2-Amino-5-carboethoxy-4-hydroxypyrimidine.

INTRODUCTION

Our group reported pyrimidine derivatives as one of the first T-type calcium channel blockers [1,2]. The pyrimidine ring is pharmacologically important functional group especially in the broad fields of cardiovascular and psychotic drugs [3-5]. The pyrimidine group is recently been reported with numerous pharmacological applications emphasizing the direction of medicinal chemistry field and significance of heterocyclics [6-10]. Though it is believed that pyrimidines are less utilized as pharmacological synthons compared to other rings like dihydropyrimidine [11,12], pyridines [13,14], furons [15,16], thiophenes [17], thiazides [18], *etc*., the evolution of newer medicinal chemistry leads are limited [19]. This is because the pyrimidine synthesis reactions usually result in lower reactions yields compared to other heterocyclics [20,21]. The recent spurt in interest of pyrimidine compounds necessitates development of viable synthetic methods. A simple condensation reaction of acetoacetate or malonate with guanidine compound resulted in substituted pyrimidine. This ring cyclization reported to have a rate limiting step and there are few homogeneous and heterogeneous catalysts reported for the same. Though these catalysts often result in improved yields [22,23], cost of the catalyst, difficulty in isolation, presence of traces by catalyst in final product *etc*., prohibit their use in bulk drug or medicinal chemistry synthesis [24-27]. The nanomaterial based catalysis is not reported for pyrimidine synthesis and our group has recently reported that Fe_3O_4@SiO_2 acts as an inert support in some organic reactions. This inert solid support catalyst was shown to give significantly improved yields for reactions involving ring cyclizations [23,28,29]. The mechanism seems to be intermediate stabilization due to its interaction with silica surface. We have reported a similar mechanism for isoxazoline formation and the improved yields seem to be specific for heterocyclics [23]. In this paper, we reported the synthesis of substituted pyrimidine synthon using 40 nm sized Fe_3O_4@SiO_2 as "near homogeneous catalyst" and the improved yields observed has possible applications in bulk drug and API synthesis.

EXPERIMENTAL

All chemicals of laboratory grade and are procured from SD Fine Chemicals India. The solvents are of analytical grade and are from Merck Pvt. Ltd. and used as such without further purification. Initial analysis of product identification was done with comparison IR spectra with commercial reference compound. The IR spectra recorded on Shimadzu IR affinity-1, Model No. 311646. The melting points are the average of three readings and recorded in a locally bought instrument.

Synthesis of silanized magnetite nanoparticles (Fe_3O_4@SiO_2)

Synthesis of Fe_3O_4 nanoparticles: In a 1 L three neck round bottom flask, 8.5 g of sodium nitrate, 5 g of sodium hydroxide, were dissolved in 500 mL of distilled water and the mixture was degassed with argon gas for 0.5 h at 90 °C on water bath (solution 1). In separate beaker 6.95 g of iron(II) sulfate was dissolved in 50 mL of distilled water having 10 mM H_2SO_4 and filtered it (solution 2). The $NaNO_3$/NaOH mixture (solution 1) was cooled to below 60 °C, then the iron sulfate filtrate (solution 2) was added slowly with constant flow rate while continuing argon gas purging. The nanoparticle formation was observed as black colour formation in solution. After few minutes the argon gas aeration was removed and reaction mixture was heated for 4 h at 90 °C under inert atmosphere (preferably argon). The hot magnetite nanoparticle mixture was poured to 1 L beaker carefully, discarded the supernatants and Fe_3O_4 particles were collected with the help of external barium ferrite magnet. The magnetic particles were washed with distilled water till pH of supernatant reached 7.

Silica (SiO_2) functionalization of Fe_3O_4 nanoparticles: The silica functionalization of Fe_3O_4 was done by sonication of TEOS in ethanol-water at pH 12. In a 1 L beaker 500 mL of ethanol/water (4:1) was mixed well and pH adjusted to 12 using NaOH. The magnetic nanoparticles were suspended ethanol/water mixture and sonicated for 1 h. To the above sonicated mixture 5 mL of TEOS was dissolved in 20 mL ethanol and added slowly throughout the solution. The sonication continued for more than 1 h and mixture was allowed to settle down. The silica coated magnetic particles are collected under external magnetic field and the supernatants were discarded. The Fe_3O_4@SiO_2 are washed with distilled water and continued till the pH of solution reaches around 7.

Washing of Fe_3O_4@SiO_2 to remove iron salts: The silica coated magnetic nanoparticles synthesized by this process could have traces of iron ions. To obtain pure silica functionalized magnetic nanoparticles, the traces of ferrous and ferric ions elimination was done by a) Fe_3O_4@SiO_2 particles were mixed with 100 mL of 0.3 M trisodium citrate at pH 6 in round bottom and refluxed for 30 min at 90 °C. Then solution was cooled, washed with distilled water 3-4 times and silica coated magnetite particles were separated using external magnet. b) The particles which are washed in above process was suspended in 100 mL of 10 mM tris, 1mM EDTA and refluxed for 0.5 h at 90 °C on water bath. The Fe_3O_4@SiO_2 was separated using magnet and washed till the pH reaches 7. The silica functionalized magnetic nanoparticles were stored in distilled water and prior to use, the particles are separated with help of magnet and dried overnight under oven. The characterization of silica coated magnetic nanoparticles was performed by using IR spectroscopy, SEM, TEM and EDS.

Synthesis of 2-amino-5-carboethoxy-4-hydroxypyrimidine with Fe_3O_4@SiO_2: In a 20 L flask 1.070 Kg of KOH was dissolved with 10 L of distilled water, stirred to get clear solution and 5 g of silica functionalized magnetic nanoparticles (Fe_3O_4@SiO_2) was added. To the above mixture 2 Kg of guanidine carbonate was added and stirring is continued for dissolution. Temperature of the reaction mixture was maintained around 20 °C. Diethylethoxy methyl melonate (2.4 kg) was added drop-wise over 3 h, the temperature of reaction mixture was raised from 20 to 35 °C. A yellow solid was precipitated and reaction was cooled. Then with the help of external magnet Fe_3O_4@SiO_2 particles was removed, particles were washed with acetone and dried. The reaction mixture was cooled to 0-5 °C and pale yellow colour thick mixture was filtered and washed with ice cold water and padded well. The product was recrystallized from ethanol/water and dried in oven at 40 °C. The dried product is divided into two parts and recrystallized in 15 L of 60 % H_2O/40 % EtOH. The recrystallization was hazy at first but goes clearer in appearance after 1 h of steam heating. This compound is to crystallize and first sign is observed at 39 °C and continued with slow cooling. The process was done for both the parts and dried under vacuum oven. Yield: 95 % (**Scheme-I**).

Synthesis of 2-amino-5-carboethoxy-4-hydroxypyrimidine without Fe_3O_4@SiO_2: The compound is synthesized as exactly same as mentioned above without using Fe_3O_4@SiO_2 particles Overall yield: 70 %. Mass: 184.07 (M+1); Anal. calcd. for C, 45.90; H, 4.95; N, 22.94; O, 26.20; ^{1}H NMR:1.38 (1H, t), 4.3 (2H, q), 8.5 (1H, m), 7.2 (2H, s), ^{13}C NMR: 162, 170.6, 155.8, 168.5, 61, 142.

RESULTS AND DISCUSSION

Synthesis of consistent 40 nm sized magnetic nanoparticles: Our group was involved in development of functionalized nanomaterials as a tool for reaction yield improvement [19]. The technical challenge of this process is obtaining consistent size nanoparticles. We performed about 40 different batches of silica functionalized Fe_3O_4 nanoparticles and

Scheme-I: Synthesis of 2-amino-5-carboethoxy-4-hydroxypyrimidine

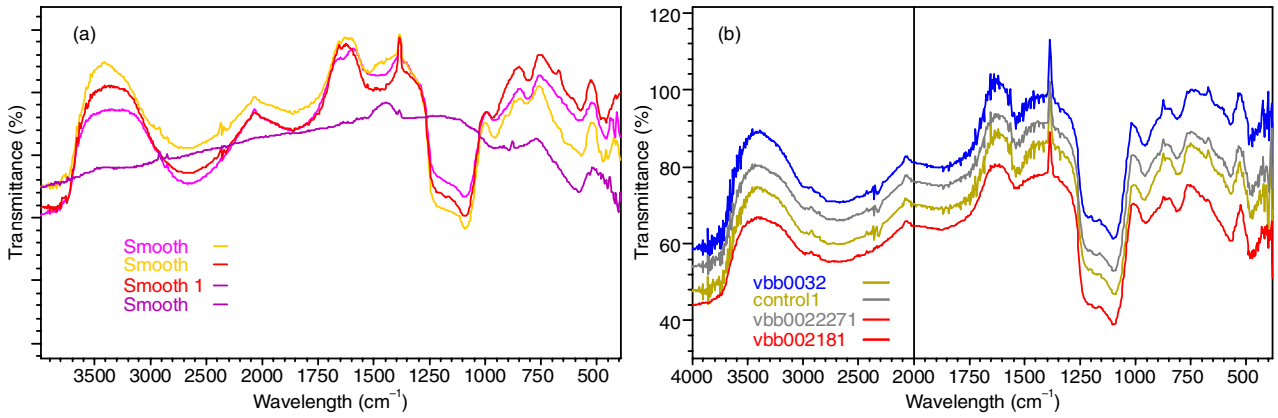

Fig 1. IR spectra of functionalized magnetic nanoparticles

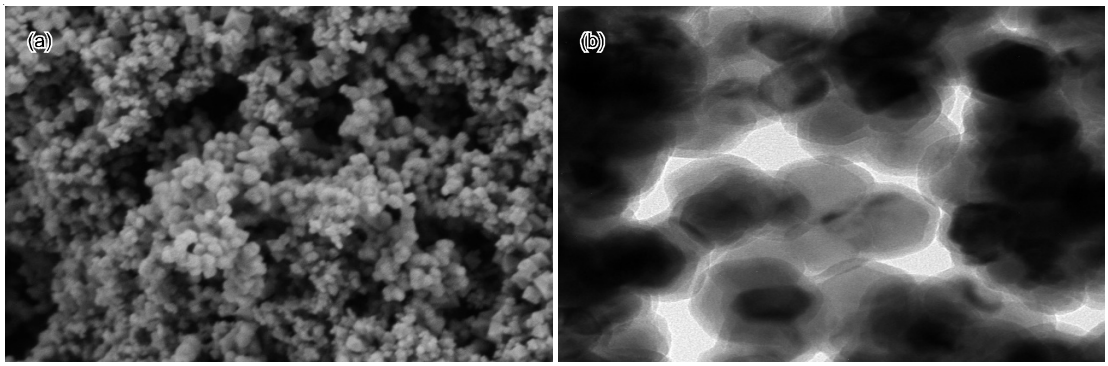

Fig 2. SEM and TEM images of Fe_3O_4@SiO_2 particles

characterized with each batch size of 100 g. Though IR is an acceptable spectral technique to know batch to batch consistency in quality control setting. The silica coated magnetic nanoparticles are characterized by IR spectra. The blue peak image (Fig. 1a) shows the non-silanized Fe_3O_4 particles. Three characteristic peaks at 1250-1100 cm^{-1} suggested that the silica coating on Fe_3O_4 (silanization) and the another image (Fig. 1b) shows batch to batch consistency of silica coating on magnetic nanoparticles. However, techniques like SEM and TEM offer more in depth analysis of the same. The SEM image (Fig. 2a) shows the 40 nm sized particles and TEM image (Fig. 2b) shows the coating of silica is uniform on Fe_3O_4 particles. The Fe_3O_4@SiO_2 particles are not calcinated and the consistent quality of particles is reflected in the data presented. Calcination is one of the major cost bottle neck for their use in process chemistry due to low yield and high cost. Interestingly 40 nm size seems to be optimal in removing the need for calcination and their catalytic properties of particles is supreme at this particle size. The EDS spectra (Fig. 3) shows that particles are synthesized by modified Stober method have only Fe, O and Si as elements. The elemental ratio state that the Fe_3O_4 particles are coated by silica. All multiple batches synthesized during this study have similar elemental ratio indicating the batch to batch consistency.

Catalytic surface area: The magnetic nanoparticles synthesized by modified Stober process yielded a Gaussian distribution of particles of the size ranging from 30 to 200 nm. The maximum number of particles are found to be around 40 nm in size and the catalytic surface area was calculated based on cube size of the particles. The catalytic surface area is

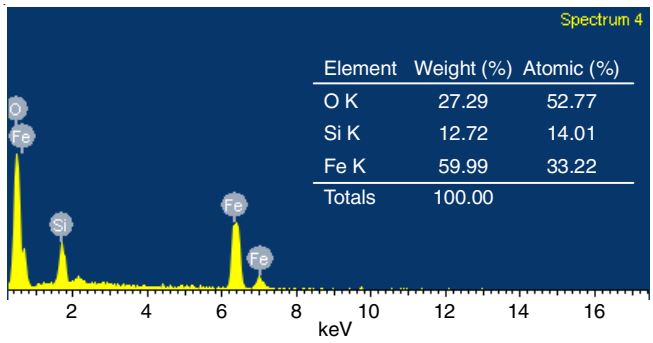

Fig 3. EDS spectra of Fe_3O_4@SiO_2

found to around 64000 nm^3 per particle. A typical reaction used about 5 % (w/v) of particles and the mechanism of catalysis is improved because of surface area. As reported earlier [1,2], the mechanism of cyclization of heterocyclics *via* Fe_3O_4@SiO_2 seems to be interaction of acidic surface with heteroatoms thereby stabilizing the intermediate. The mechanism reported for isoxazole [19] had rate limiting step and incidentally pyrimidine reaction through Knoevenagel condensation reported here also possess the same. The silica surface area was adequate at 5 % w/v as shown in Table-1 and increasing further catalyst condensation did not result in yield improvement. But, lowering the catalyst concentration to < 1 % has lowered the catalytic efficiency and Table-1 shows the yields. It was observed and reported in literature that heterogeneous catalysis often requires certain % of catalyst by (w/v) and of theoretical catalytic surface area can be only be indicative [20-23].

TABLE-1
STUDY ON DEPENDENCE OF CATALYST (% w/v) IN OVERALL REACTION YIELD

S. No.	Concentration ratio of catalyst (%)	Reaction yield (%)
1	< 1	77
2	1	85
3	2	87
4	3	91
5	5	95
6	7	90
7	10	82
8	Without catalyst	76

Synthesis of pyrimidine by Knoevenagel condensation: Dihydropyrimidines and pyrimidines are known for then T-type calcium channel blocking activity [1,2]. The pyrimidines have become a choice class of their medicinal chemistry because of stability towards oxidation compared to dihydropyridines. The preferred synthetic route for pyrimidines is through Knoevenagel condensation of an aldehyde or ketone and an acetoester. The reaction yields decreased due to the water molecule generated in the reaction is an inhibitor of the reaction. *in situ* removal of water and moisture absorbing materials are the two options [25-27], which were often observed with minimum success and inconsistent yields [25-27]. Recent mechanism studies had suggested that moisture was not the real problem and the catalysts offering more surface area have proven to be more useful [28-31]. The use of Fe_3O_4@SiO_2 was first of its sort and near doubling of reaction yield is in expected lines considering our similar observation with thiophenes, terpyridine, quinazoline and isoxazole [23].

Conclusion

2-Amino-5-carboethoxy-4-hydroxypyrimidine is a useful synthon for medicinal chemistry and we reported herein a simple and scalable synthesis for the same. The doubling of yield has represented that Fe_3O_4@SiO_2 does help in improvement of reaction yields of heterocyclics. The Knoevenagel condensation was reported to be a low yielding reaction [32,33] and we found a practical and mild way to improve the reaction yield. The Fe_3O_4@SiO_2 particles are about 40 nm size and the size of the particles does play a role in reaction improvement. The optimum catalytic ratio was about 5 % (w/v) and using less than 1 % (w/v) results in reactions yields of uncatalyzed reactions. The marginal decrease reaction yield at 7-10 % (w/v) catalyst needs further study and *in situ* decomposition of product at higher catalytic concentration needs be understood in future studies.

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