# FORMULATION AND EVALUATION OF NANOFORMULATIONS OF BICALUTAMIDE

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Abstract:

Bicalutamide (BIC) is a non-steroidal anti-androgen used for monotherapy in treating prostatecancer. The nanoparticles of bicalutamide were prepared by sol- vent evaporation method and evaluated for FT_IR, DSC, X-ray diffraction invi- tro dissolution and invivo diffusion studies. The spectra of unfunctionalized MSNs were comparatively simple and major peaks could be easily assigned. A characteristic peak attributed to BIC could be seen. The melting point of BIC was found to be 192.54 ℃ denoted by a sharp endothermic peak. The complete entrapment of drug into meso- porous carriers was confirmed by absence of any such peak in the thermogram. This pat- tern was well preserved and even after functionalization and drug loading. BIC release fromMCM-41 was highest followed by marketed formulation. It causes dose depend entre ductionin the Prostate specific antigen(PSA) levels.Ithas the potential of working wonders at all the stages of prostate cancer disease continuum. Thus, the article reavels the application of bare and functionalized MSNs as oral as well as intravenous targeted delivery agents for BIC in cancer therapy have been pro- posed in depth along with their biosafety aspects and efficacy.

**Keywords:** Nanoformulation, FTIR, DSC, In vitro dissolution, XRD

**Introduction:**

Bicalutamide (BIC) is a non-steroidal anti-androgen used for monotherapy in treating prostatecancer (1). It causes dose dependent reduction in the Prostate specific antigen(PSA)levels.Ithas the potential of working wonders at all the stages of prostate cancer disease continuum(2). BIC binds to cytoplasmic andro- genic receptors and competitively inhibits the androgenic action by producing distortion of the co-activator binding site, thereby stopping the initiation of gene transcription. It causes some central androgen blockade and not much affects the testosterone and LH levels. It is also approved for combination therapy along with LHRH analogue for treating metastatic prostate cancer. It possesses alonge rt1/2 and undergoes an extensive metabolism in liver (3). Some of the major challenges currently faced by the current cancer treatment in- clude the solubility and permeability limitations faced by most of the new chem- ical entities and anticancer agents. Another important challenge is reducing the side effects of the current chemotherapeutic treatment by aiming for tumour tar- geted therapy for cancer. The most recent advancement being “theranostics” in which simultaneous diagnosis and the therapeutic treatment is possible by incor- porating both the agents in a single carrier. BIC comes under Biopharmaceutical classification system (BCS) Class II and suffers from solubility limitations, lead- ing to dissolution and bioavailability issues.Thus,formulatinganovel drug deli- very system for this drug could open new avenues for increasing its effectiveness in prostate cancer treatment. To achieve this purpose a mesoporous silica nano delivery system was designed using MCM-41 carriers and thereafter functiona- lizing with ligands and surface moieties which help in achieving a targeted me- soporous system for the drug BIC.

The drug delivery systems were designed based on the pH stimuli based and another was basedon targeting the receptors overexpressed in cancer like folate receptors. PAA polymer was used as a pH responsive material and coated onto the surface of MCM-41 MSNs via an aminated intermediate layer. This was based on the fact that environment of cancer cells is more acidic than that of the healthy cells. Also, certain receptors are found to be over expressed in cancer- cells like folate receptors. Folic acid was used as a ligand to target the overex- pressed folatereceptorsincancer.

Thus, the article reavels the application of bare and functionalized MSNs as oral as well asintravenous targeted delivery agents for BIC in cancer therapy have been proposed in depth alongwith their biosafety aspects and efficacy.

Thefacilesynthesisandfunctionalizationstrategywasadopted.DissolutionandCaco- 2Permeability study was

performed and Pharmacokinetic data and biodistribution analysis wasnoted and cytotoxicity study was done on LNCaP and PC-3 prostate cancer cell lines. The cell death mechanism, cell killing efficiency and cellular uptake was determined. Athorough histological examination was done to adjudge their preliminary biosafety.

Materials and Methods:

Materials

Bicalutamide (BIC) was obtained as asample gratis from Intas Pharmaceuticals Ltd, (Ahmedabad, Gujarat, India). Other Chemicals required for the re4search study were purchased from S.D. Fine Chem Ltd, Mumbai.

Methods:

Synthesis of bareand functionalized MSNs

AminatedMCM-41NPswereobtainedbyreactionofMCM-41NPswithAPTESaspera re- ported method in the literature (2). Typically, 250 mg of MCM-41 was accurately weighedand transferred to a round bottom flask (RBF). The MCM-41 MSNs were dis- persed in 30 mLtoluene. Thereafter, 3.43 mL of APTES wasadded. The reaction was kept under vigorous stirringat 70°C for 12h. Thereafter, RBF was allowed **to cool slowly at RT.** The reactionmixture obtained was filtered and washed with methanol. The product obtained was dried andlabelled as MCM-41-A. Successful functionaliza- tion with APTES laid a strong foundation forfurther functionalizationwithPolyacryli- cacid(PAA). Differential scanning calorimetry (DSC) analysis: Melting point and loading were determined by DSC Shimadzu-TA60 thermal analyzer equipped with theTA60 WSsoftware.Theheatingratewaskeptat10°C/min. The existence of BIC and BIC on silica matrix in amorphous or crystalline form and complete encap- sulation was further confirmed by DSC. Absence of any melting point depression is indicative of presence of drug in non-crystalline state in pores (8). Melting points of BIC and BIC were determined individually. DSC analysis of BIC-MCM-41, BIC- MCM-41-A, BIC-PAA-MSN,BIC-FA-MSN,BIC-MCM-41,BIC-MCM-41-A,BIC-

PAA-MSN,BIC-FA-MSN was done and absence of any crystalline sharp drug peaks was ensured.

X-Ray diffraction analysis(XRD)

PANalytical model equipped with Cu K radiation beam operating at 40 kV and40 mA was used to determine low angle X-Ray powder diffraction (LXD) pattern. The struc- ture of pores was ascertained by low-angle XRD measurements. Thespectra was an in- dication ofthe intactness of the mesoporous structure. LXRD measurements were taken for MCM-41, BIC-MCM-41, MCM-41-A, BIC-MCM-41-A, PAA-MSN, BIC-PAA-MSN, FA-MSN andBIC-MCM-41,BIC-MCM-41-A,BIC-PAA-MSNandBIC-FA- MSN.

Morphological characterization was performe dusing scanning electron microscopy (SEM) (FEI-Quanta 200 operating at 20 kv) (Thermo Scientific, USA). The samples were coated withgold to make them conducting before imaging.The images were re- solved over aphotographic film. SEM and analysis was done to determine morphologi- cal and internal structure of bare MCM-41 and functionalized PAA-MSN and FA- MSNs respectively.

Dissolution Kinetics study

The % cumulative release data obtained from dissolution study was fitted to vari- ous kineticmodelsandthe bestfit wasdeterminedbasedontheR2value,AIC criterion andModelselectioncriterion.

Invitro diffusion study

Phosphate buffer saline (PBS) media of different pH like 5.5, 6.8 and 7.4 was used to determinethe in vitro release behaviour of BIC. A dialysis tube having cut-off Molecular weight (Mw) of7000 g/mol was used to fill the suspension of BIC, BIC-MCM-41, BIC-MCM-41-A,BIC-PAA-MSNs and BIC-FA-MSN. Continuous magnetic stirring was provided and sink conditions were maintained properly by replacing withdrawn samples immediately with fresh PBS of respective pH.The withdrawn samples were analysed by measuring the fluorescence intensity by Spectrofluorometer keeping affixed excitation wavelength of 260 nm and measur- ing.

Zeta potential and size determination:

Particle Z-average size and charge were measured using dynamic light scattering (DLS) and electrophoretic mobility measurement srespectively, usingthe Malvern Ze-tasizer Nano ZS (Malvern instruments, Malvern, UK). Zeta potential indicates the sur- face residual charge of theparticles. The zeta potential measurements were done for MCM-41, MCM-41-A, PAA-MSN,FA-MSNanddrugloadedMCM-41,MCM-41- A,PAA-MSNandFA-MSN.

In vitro release study:

The drug release pattern was determined and cumulative drug release was calculated for all the mesoporous formulations of BIC. For formulations to be administered by oral route, dissolution study was performed. Additionally simulated and biorelevant media was used tostudy the effect of enzymes and food on drug release. For parenter- al formulations, in vitrodiffusionstudywasperformedatdifferentpHvaluesu-singPBSmedium.

Invitro dissolution study

Dissolution study was performed using Veego USP type II dissolution apparatus in 900 mLdissolution media at 50 rpm maintaining temperature of dissolution medium at 37 ± 0.5°C. Thein vitro release study was performed for plain BIC (API), MF, BIC- MCM-41 and BIC-MCM-41-A in acetate media (pH 4.6), simulated gastric fluid (SGF) (pH 1.2) and simulated intestinalfluid (SIF) (pH 6.8) containing pepsin and pancreatin respectively. Enzyme free SGF (pH 1.2)and SIF (pH 6.8) were also taken to study the presence of any interaction between hard gelatincapsule shell and amine group of MCM-41-A and whether it has any effect on the release ofBIC. The drug re- lease pattern was also studied in the presence and absence of food as well.Hence, the fed and fasted state simulated gastric and intestinal media were prepared (FaSSGF,FeSSGF. FaSSIF, FeSSIF) for this purpose. The composition of all media is summarized inTable 6.1 (23, 24).The powder was filled in the hard gelatin capsule shell prior to dissolutionstudy. 5 mL aliquots were withdrawn at 5, 10,15,20,30,45,60,90,120,180,240,320 and 360 minintervals.The withdrawnsamples- were filtered through0.45µPVDF filter membrane andanalysed by UV spectrophoto- meter at 285 nm. Sink conditions were maintained throughout the study by adding an equivalent amount of fresh medium as that of withdrawn sample.

Dissolution Kinetics study

For the purpose of quantifying the differences in the release profiles of BIC formula- tions. Thedrug release data from bothMCM-41 and MCM-41-A nanoparticles was fit- tedto variouskinetic models and the best fit was determined. Different parameters by fitting the experimentaldata to different release models were calculated. Criteria for judgement on **best model included lowest AIC (Akaike information criterion), highest MSC (Model selection criterion) and Regression values(R2)(25). The various release models to which dissolution data were fitted include zero order, first order, Higuchi, Weibull, Hixon-Crowell and Korsmeyerpeppas model(26).**

**Invitro diffusion study**

In the in vitro drug release study, a suspension of BIC, and BIC-MCM-41, BIC- MCM-41-A,BIC-PAA-MSN and BIC-FA-MSN was filled into a dialysis tube (cutoff Molecular weight(Mw) = 7000 g/mol for PAA-MSNs) and (cut-off Mw=3500 forFA- MSNs). Further, the bagcontaining filled dispersion was then immersed into 100 mL

In vitro dissolution study

Veego USP type II paddle apparatus was used to perform the dissolution study in 1000 mLdissolution media at 50 rpm maintaining 37 ± 0.5 °C temperature. Thein vitro disso- lutionstudy was performed for BIC, Marketed Formulation (MF), BIC-MCM-41 and BIC-MCM-41-A in various media viz water (with addition of 0.5% SLS), FaSSGF, FeSSGF, FaSSIF, andFeSSIF (Fast and Fed state biorelevant media) to study the drug re- lease pattern from non-functionalized

Results and Discussion

Fourier Transform Infra Red (FT-IR)spectroscopystudies

FT-IR spectra (Figure. 1) provided proof of the manner in which successful syn- thesis and functionalization of MCM-41mesoporous silica nanoparticles pro- ceeded. The spectra of unfunctionalized MSNs was comparatively simple and major peaks could bee asilyassigned. In a characteristic peak attributed to BIC could be seen. The cyanide and amide carbonyl groups were denoted by peaks obtained at 2227cm-1,1685cm-1and3341cm-1respectively.

Aromatic stretching due to phenyl group was observed at 708 and 833 cm-1. Characteristic cyano and carbonyl group peaks were seen at wavenumbers 2230 and 1689cm-1respectively. In case of non-functionalized MCM-41(b)&(c) a syn- thesized and calcinated MCM-41could be distinguished by non-existence of C-H stretching (2954 and 2854 cm-1) and deformation vibrations at 1452cm-

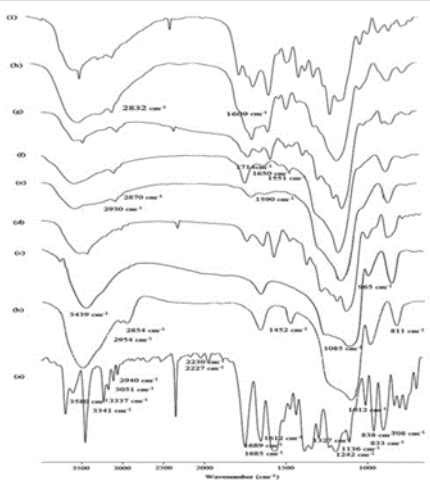

Figure.1.FT-IRSpectra(a)BIC(b)AsynthesisedMSN(c)MCM-41(d)BIC-MCM-41 (e)MCM-41-A(f)PAA-MSN(g)BIC-PAA-MSN(h)FA-MSN(i)BIC-FA-MSN

Differential scanning calorimetry (DSC)analysis

DSC was used in determining the melting point of the drug BIC and investigate complete entrapment at a preliminary level. The melting point of BIC was found to be 192.54 ℃ denotedby a sharp endothermic peak (Figure 2). The complete en- trapment of drug into mesoporous carriers was confirmed by absence of anysuch peak in thethermogram.

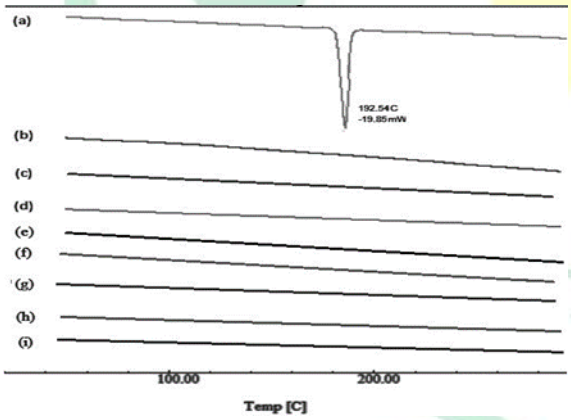


**Figure.2.**DSCthermogramsof(a)CrystallineBIC(meltingpointofdrug),(b)MCM-41 (c) BIC-MCM-41,(d)MCM-41-A(e)BIC-MCM-41-A(f)PAA-MSN(g)BIC-PAA-MSNh)FA-MSN(i)BIC-FA-MSN

**X- Ray diffraction analysis (W-XRD)**

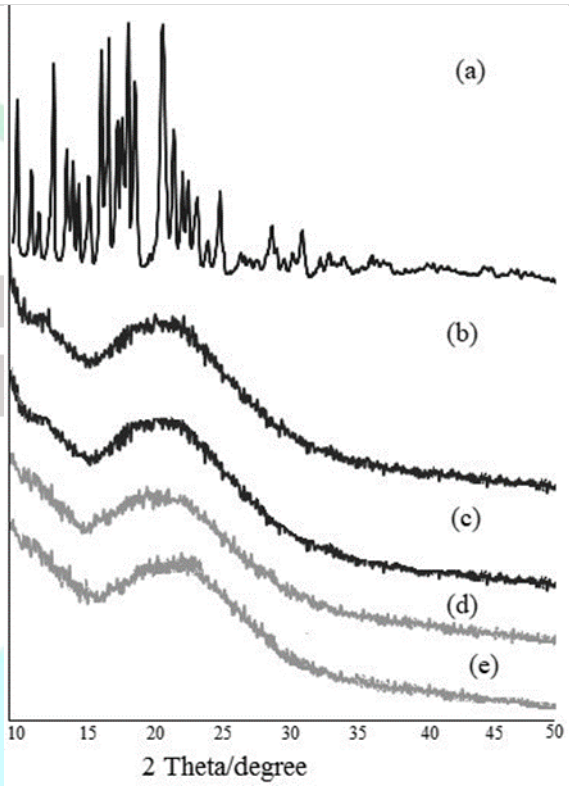


**Figure.3.WAXRDspectra(a)BIC(b)BIC-MCM-41(c)BIC-MCM-41-A(d)BIC-PAA-MSN(e) BIC-FA-MSN**

Crystalline nature of BIC was justified by the sharp and highly intensified peaks obtained in the wide angle XRD spectra (Figure 3). The position of the peaks identified in order of their ascendingthetavalues are 12.29,18.15,23.29,24.11,29.1,29.30,31.45,and34.82θ.
When we see the Wide-angle spectra of drug loaded MSNs, no clear intense peak is visi- ble. This indicates the conversion of crystalline BIC to amorphous form after entrapment into MSNs. This fact could play a major part in dissolution enhancement of drug. The spectra of drug loadedbare and functionalized MSN sindicated a complete entrapment of drug in to the mesopores.

**Estimation of drug loading efficiency**

The pore size and pore volume also contribute in determining the maximum amount of drugwhich can be entrapped in MSNs. A slight reduction in loading per-centage from MCM-41 topostfunctionalizationinMCM-41-Awasobserved.

Additionally, the TGA data also prove the thermal stability of silica materials for proposed potential application assited irecting carrier forBIC.TheTGA data exhi- bited extent of grafting as 4% incase of MCM-41-A,20.19 % in PAA-MSNs and 23.75% incase of FA-MSNs

**Invitro dissolution study**

Drug release study was carried out in 1000 mL water with 0.5% SLS to deter- mine the releasepattern by calculating the cumulative drug release at different time points.BIC release fromMCM-41 was highest followed by marketed formu- lation. However, a controlled and sustainedbehaviourofdrug releasewas obtained in case of amine functionalized MSNs. Additionally, fast and fed state gastrican- dintestinalmediawereusedtostudyifthereisanyeffectoffoo dondrug release from all the formulations (7). The drug release was almost similar in case ofboth fast and fed state conditions and hence it could be concluded that presence of food did no- talter drug release (Fig 7.7). Thus,the medication could be taken either ways emptyor aftermeal, it wouldn't affect the absorption or release. Further, various dissolution kinetic modelswere applied on the obtained %CDR data. Of the vari- ous models applied he highest regressioncoefficient and MSC value along with lowest AIC criteria was best fit in Weibull model in caseof MCM-41 type meso- porous matrix (8) (Table 7.6 and 7.7). Whereas, in case of MCM-41-Amatrix Hi- guchi model was found to the best fit satisfying all the three criteria

Invitrodiffusionstudy The pH responsive based release was investigated for BIC, BIC-MCM-41, BIC- MCM-41-A,BIC-FA-MSN and BIC-PAA-MSNs at PBS media of different pH viz, 5.6, 6.8 and 7.4 as afunction of time(Fig 7.8).It was observed that BIC-PAA- MSNs exhibited a highly pH responsive behavior. Drug release fromPAA-MSNs wasfound to varying inversely and decreased with increase in pH.Maximum BIC release at 72 h was observed at pH 5.6 with percentage cumulative release being 89.92±0.65%. The release at pH 6.8 and 7.4 was found tobe 68.85±0.98 % and 30.72±0.72 % respectively. At lower pH PAA gets completely protonated lead- ing to enhanced release of BIC due to weakened interactions. However, for BIC- MCM-41NPs the release reached 98.72±0.82% at pH 5.6 within 48 h. Though, the release was up to 1.23times faster in case of drug loaded bare silica when compared to BIC-PAA--MSN, no pHdifferentiating effectwas seen, understably- duBIC absence of any pHresponsivemoiety onbare MCM-41.Further, PAAcoat- ing could also be held responsible for theslow release ofBIC. A slight pH res- ponsive behaviour was seen in case of BIC-MCM-41-A and BIC-FA-MSN but- not as major as PAA-MSN.The releaseof BIC from PAA-MSN and MCM-41- Awasfasterthanthat ofPAA-MSN. However, at higher pH like 7.4 it might be

**Conclusion:** The results obtained were suggestive of successful BIC encapsulation into the MSN skelBICnboth bare and surface functionalised. A significant enhancement in its dissolution rate andbioavailability with 3.12 and 2.61 times respectively as compared to BIC was obtained for BIC-MCM-41. Whereas the results obtained were 1.86 and 1.38 respectively for BIC-MCM-41-A.This could aid in dose re- duction and enhanced efficacy at thesametimeovercoming thesolubility limita- tions.The increment in dissolution and bioavailability could also lead to dosere- duction.The permeability of BIC-MCM-41NPs and BIC-MCM-41-ANPs were en- hanced 4.66 and 2.71 times respectively with respect to BIC. The apoptotic assay revealed that BIC-PAA-MSN and BIC-FA-MSN were able to induce a higher

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