

Original Research Article

Keratin/alginate microspheres as a novel pH-dependent polymer for colon-targeted delivery of dexamethasone for the management of inflammatory bowel disease

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Abstract

Purpose: To develop keratin/alginate microspheres loaded with dexamethasone for managing inflammatory bowel disease (IBD), enabling targeted delivery to the colon and minimizing systemic side effects to improve therapeutic efficacy.

Methods: Microspheres were prepared using ionotropic gelation method. Air-dried microspheres were evaluated for entrapment efficiency, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), sieve analysis, scanning electron microscopy (SEM), in vitro dissolution, and stability studies.

Results: Microsphere entrapment was between 86 to 98 %. The SEM revealed discrete particles with rough surfaces while FTIR and DSC results did not show any interaction among components in the microspheres. Cumulative drug release was < 30 % after 2 h at acid pH and 72 – 94 % after 12 h in all formulations.

Conclusion: Keratin/alginate microsphere demonstrates its potential as a suitable pH-sensitive composite indicating a promising platform for colon-targeted dexamethasone delivery.

Keywords: Keratin, Alginate, Microsphere, Colon-targeted delivery, Dexamethasone, pH-dependent polymer

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic disorder in which the intestines become inflamed because of pathogens, metabolic disorders, physical substance exposure, etc. Dexamethasone is used in the treatment of inflammatory bowel disease (IBD). Orally administered dosage forms pass through the complex gastrointestinal tract (GIT; acid and

enzyme degradation) and may undergo first-pass metabolism in the liver, thus reducing the concentration that is available at systemic circulation. This invariably also affects the proportion of drugs that reach the colon. One of the strategies for targeting orally administered drugs to the colon is the use of microparticulate systems [1]. Microspheres are micromatrix systems comprising a homogeneous mixture of active substance (a drug) and polymer. They

have been used to target drugs to sites, sustain drug release, decrease the frequency of administration, and improve patient acceptance and compliance [2]. Their micro-size nature allows them to easily traverse the GIT.

Keratin is a natural, biodegradable protein polymer that is insoluble at acidic pH but increases in solubility as the pH rises above neutral. The novelty and significance of using keratin in microsphere formulation lie in the utilization of keratin as a biocompatible and biodegradable polymer [3], reducing the risk of adverse reactions and enabling natural degradation of keratin microspheres. Moreover, keratin has pH-dependent properties [4] thus facilitating the targeted release of drugs minimizing exposure to healthy tissues and reducing side effects. This innovative approach ensures the stability of microspheres in acidic environments, sustains drug release and enhances bioavailability in the colon for effective treatment of colon diseases, such as inflammatory bowel disease (IBD). Furthermore, keratin offers a cost-effective alternative to existing drug-delivery polymers and has the potential for large-scale production [5]. By leveraging keratin's unique properties, this platform provides targeted, sustained, and controlled drug delivery, improving patient compliance and treatment outcomes.

Over the years, some formulations containing keratin have been reported. Wilson *et al* [6] reported the microencapsulation of an oil-soluble dye (Nile red) using keratin-chitosan crosslinked with glutaraldehyde in cosmetics formulation. The formulation showed stable microcapsules with enhanced dye release. A study of the combination of keratin/HPMC also showed the sustained release profile of diclofenac matrix tablets was improved compared to either ingredient alone [7]. Yin *et al* [8] fabricated rhodamine-casted films using keratin. The films were pH-responsive and showed controllable drug release. This work aims to investigate keratin and alginate microspheres for colon-targeted delivery of dexamethasone.

EXPERIMENTAL

Materials

Keratin was extracted from domestic chicken feathers (*Gallus gallus domesticus*) collected from a local poultry farm in Anambra State (latitude - 6.33815 and longitude - 5.62575), Nigeria in April 2021. Dexamethasone (CAS no. 50-02-2, ≥ 98%) powder was purchased from Safety Pharmaceuticals, Nigeria. Sodium

alginate was a gift from Sanofi Bio- Bio- Industries, sodium hydroxide (BDH UK), hydrochloric acid and calcium chloride (Loba Chemie, India), Tween 80 (polyethylene glycol; PEG) (Sigma Aldrich, Hamburg, Germany).

Keratin extraction

Disposed chicken feathers from poultry were collected and keratin was extracted from feathers using the alkaline-hydrolysis method previously reported [4]. The keratin used in this study was the sample from this previously reported method.

Pre-formulation studies

Ionotropic or ionic gelation method was used to make the dexamethasone microspheres. A preliminary formulation was done to select the optimal combination ratio of keratin and sodium alginate. Polymer ratios that yielded particles with small sizes and less agglomeration/aggregation, as shown in Table 1, were selected for further production.

Table 1: Variables of keratin/alginate blank microsphere

Property	Material	P1	P2	P3	P4
Polymer	Keratin (%)	0.5	1	2	1
	Sodium alginate (%)	0.5	1	1	2
Gelling solution	calcium chloride (%)	2	2	2	2
Surfactant	tween 80 (mL)	0.2	0.2	0.2	0.2

FORMULATION STUDIES

Preparation of core material

Core materials for the formulation of different batches of microspheres (MS) were prepared using the formula in Table 2. In M1 - M5, a dispersion containing keratin and alginate was prepared by dissolving a double concentration of keratin and alginate in 100 mL of phosphate buffer and distilled water, respectively. The two polymer solutions were mixed to form a composite containing the required amount of each polymer. Dexamethasone (500 mg) was added, and the mixture was stirred using a magnetic stirrer (SH-2 magnetic stirrer, UK) until a homogenous solution was formed.

Preparation of gelling solution

Two grams (2.0 g) of calcium chloride dehydrate was dissolved in 100 mL of distilled water and 0.1 mL of polysorbate (Tween 80) was added to lower the surface tension of the solution in order to facilitate the production of spherical droplets.

This was used as the gelling solution for M1 – M5. In M6 - M9, the gelling solution contained the required amount of keratin and calcium chloride in 100 mL of gelling solution.

Microsphere production

Ionotropic gelation method reported by Arhewoh *et al* [10] was used. A 10 mL syringe containing the dexamethasone and keratin/alginate composite and fitted with a gas-chromatographic (GC) blunt-tipped needle (21G) was attached to a syringe pump (Fisherbrand™ 780100I, USA). The 10 mL samples from M1 - M5 were extruded into beakers containing 50 mL of calcium chloride solution, while M6 - M9 was similarly extruded into the solution of keratin and calcium chloride to form spherical droplets. Microspheres were left in the solution for 2 minutes for complete reaction, gelling and hardening and then washed twice with distilled water and air-dried. The microspheres formed are shown in Figure 1.

Evaluation of keratin/alginate microsphere

Entrapment efficiency

The quantity of microsphere equivalent to 50 mg dexamethasone was crushed and dissolved in 100 mL of 0.1 M phosphate buffer (pH 6.8) in a volumetric flask. The dispersion was kept overnight to allow a complete dissolution of microsphere (MS) containing the entrapped drug and was filtered using Whatman filter paper number 4 to obtain clear solution. Drug content was analyzed spectrophotometrically at 303 nm. Entrapment efficiency (EE) was calculated using Eq 1 while drug loading (DL) was calculated using Eq 2.

$$EE (\%) = (C/TC)100 \quad (1)$$

$$DL (\%) = (C/W)100 \quad (2)$$

where C is the entrapped drug concentration, TC is the theoretical concentration and W is the weight of dried microsphere.

Table 2: Composition for dexamethasone microspheres formulation

Property	Ingredient	Formulation code								
		M1	M2	M3	M4	M5	M6	M7	M8	M9
Core material	Dexamethasone (mg)	500	500	500	500	500	500	500	500	500
Polymer solution	Keratin (%)	-	0.25	0.5	0.75	1	-	0.25	0.5	0.75
	Sodium alginate (%)	2	1.75	1.5	1.25	1	2	1.75	1.5	1.25
Gelling ion solution	Calcium chloride (%)	2	2	2	2	2	2	2	2	2
Surfactant	Keratin (%)	-	-	-	-	-	0.25	0.5	0.75	1
	Tween 80 (mL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

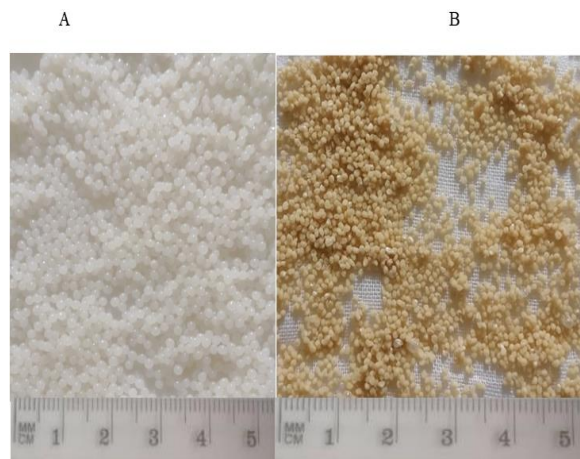


Figure 1: (A) microspheres containing sodium alginate alone, (B) microspheres containing keratin

Micromeritics

Flow rate and angle of repose

This was carried out using the funnel method described by Carstensen and Chan [9]. A known weight (w) of the microspheres was poured into a glass funnel clamped to a retort stand and hanging about 5.0 cm above a flat surface. The plug was removed from the tip of the funnel and the time (t) in seconds taken for microspheres to completely flow through the funnel orifice was recorded. Furthermore, the height of the heap formed by granules and the diameter of the heap base were recorded. The process was carried out three (3) times, and the average determination was computed. Flow rate and angle of repose (θ) were calculated using Eq 3 and Eq 4.

$$\text{Flow rate} = w/t \quad (3)$$

$$\theta = \tan^{-1}h/r \quad (4)$$

where h is the height of the conical powder heap, and r is the radius of the circular base.

Bulk density (BD) and Tapped density (TD)

A weighed amount (m) of the microspheres was transferred into a 100 mL measuring cylinder and the volume occupied by them (bulk volume; v_1) was recorded. The measuring cylinder was then tapped 100 times on a horizontal base using a locally fabricated tapping machine and the volume occupied after tapping (tapped volume; v_2) was recorded. The average of triplicate values was determined and bulk and tapped densities were calculated using Eq 5 and Eq 6.

$$\text{Bulk density} = m/v_1 \dots\dots\dots (5)$$

$$\text{Tapped density} = m/v_2 \dots\dots\dots (6)$$

Carr's compressibility index and Hausner ratio of keratin-alginate MS were calculated using Eqs 7 and 8, respectively.

$$\text{Carr's index (CI)} = (\text{TD}-\text{BD}/\text{TD})100 \dots\dots\dots (7)$$

$$\text{Hausner's ratio (HR)} = \text{TD}/\text{BD} \dots\dots\dots (8)$$

Infrared spectroscopy

The microspheres were analyzed using Fourier transform infrared spectroscopy (FTIR). About 1 - 2 mg of dried microsphere was crushed and blended with 200 mg of potassium bromide (KBr) powder and compressed into pellets using a Tablet press. The pellet was placed in the sample compartment. Spectra were recorded on a BX, Perkin-Elmer FT-IR spectrometer and scanned at an IR range of 4000 - 800 cm^{-1} , with a resolution of 4 cm^{-1} (Beaconsfield Bucks, England).

Differential scanning calorimetry (DSC)

Some quantities of microsphere were pulverized and put in an aluminium pan. The pan was placed in the heating compartment of the DSC and heating of the sample was carried out at 10°C/minute from 30 to 500 °C under nitrogen as the purge gas, at a flow rate of 70 mL/minute using the Tryte instrument, China.

Morphological characterization of keratin/alginate microsphere

The shape and surface analysis of the microspheres was determined by scanning electron microscopy (SEM; Phenom PRO-X SEM, Netherlands) equipped with an Oxford x Max 50 silicon Drift energy dispersive X-ray detector at 15 KV under high vacuum. Microspheres were mounted on a stub with

adhesive carbon and coated with 20 nm carbon with a quorum Q150R ES mini sputter coater.

In vitro dissolution study

Microspheres equivalent to 50 mg dexamethasone were loaded into the dissolution apparatus, thermostated at 37 ± 0.5 °C and stirred at 50 rpm. The dissolution study was carried out in a biphasic medium for 24 hours. Firstly in 0.1 M HCl (pH 1.2) for 2 hours and then continued in phosphate buffer (pH 6.8; to simulate the colon pH) until 24 hours. At hourly intervals, aliquots of 5 mL of sample were withdrawn and replaced with an equal volume of the dissolution medium to preserve a sink condition. Samples withdrawn were then filtered and analyzed spectrophotometrically at 303 nm against the blank phosphate buffer pH 6.8.

Kinetic data analysis

The data obtained from *in vitro* release studies were subjected to various release models; namely; zero order, first order, Higuchi and Korsmeyer-Peppas. Kinetic studies were used to determine the model that best represents dexamethasone release.

Stability studies of microspheres

Microspheres were packed in suitable containers and stored at ambient conditions of temperature and relative humidity (28 – 30 °C / 75 % RH) for 3 months on a shelf. The microspheres were evaluated for the percentage entrapment efficiency at specified periods (1, 2 and 3 months) to assess the stability.

Statistical analysis

Analysis was performed using IBM SPSS Version 23. All experiments were carried out in triplicates and the mean values were expressed with their standard deviations (SD). The difference between means was determined using one-way analysis of variance (ANOVA) at a significant value of $p > 0.05$.

RESULTS

Characteristics of blank keratin/alginate microsphere

The result from the blank microsphere shows that all particles were discrete without agglomeration. There was no significant difference in the microsphere size ranges ($p < 0.05$) but there was variation in the appearance of the microspheres. The P1 particles showed no

spherical shapes that formed mushy agglomerated/aggregated particles which were irregular when they are wet but became large, flattened, or collapsed and had irregular to shapeless forms when dried. Particles in P2 group were smaller in size formed no agglomeration and maintained the same shape during and after drying but were irregular and collapsed. The P3 particles did not show any agglomeration, were less spherical, and less collapsed, while P4 particles formed regular round to spherical particles that were distinct or separate without agglomeration. P4 was, therefore, selected as the polymer ratio concentration to be varied in the formulations of other batches M1 – M9; keratin was varied between 0 - 1 %, while alginate was between 1 – 2 %.

Entrapment efficiency of keratin/alginate microsphere formulation

The values for drug content, percentage entrapment efficacy (EE) and drug loading are shown in Table 3. The microsphere has a

percentage (%) EE between 86 – 98 %. Drug loading in the microsphere was between 11 to 23 %.

Table 3: Entrapment efficacy of keratin/alginate microsphere

Batch	Entrapment efficiency (%)	Drug loading (%)
M1	86	11
M2	91	13
M3	94	20
M4	98	21
M5	98	23
M6	88	12
M7	93	18
M8	97	19
M9	98	21

Micromeritics/bulk properties of the formulations

The values of the micromeritics/ bulk properties of keratin/alginate MS are shown in Table 4.

Table 4: Micromeritic properties of keratin/alginate microsphere

Code	Flow rate (g/sec)	Angle of repose (°)	Bulk density (g/mL)	Tapped density (g/mL)	Carr's index (%)	Hausner's ratio
M1	4.20±0.00	7.40±0.10	0.60±0.01	0.70±0.01	14±0.12	1.1±0.01
M2	5.00±0.03	8.40±0.60	0.51±0.01	0.61±0.02	16±0.12	1.2±0.02
M3	4.36±0.02	8.96±0.05	0.51±0.01	0.61±0.01	16±0.12	1.2±0.01
M4	4.42±0.03	10.03±0.05	0.41±0.01	0.51±0.02	20±0.10	1.2±0.01
M5	4.22±0.02	14.14±0.22	0.40±0.01	0.50±0.01	20±0.06	1.2±0.01
M6	4.28±0.01	9.30±0.10	0.61±0.01	0.71±0.01	14±0.10	1.1±0.01
M7	4.40±0.01	13.56±0.05	0.50±0.01	0.60±0.00	16±0.06	1.2±0.00
M8	3.19±0.01	12.23±0.11	0.50±0.01	0.60±0.01	16±0.12	1.2±0.01
M9	3.13±0.02	8.20±0.10	0.51±0.01	0.61±0.01	16±0.10	1.2±0.01

Note: Results are presented as mean ± SD of triplicate determination. *angle of repose* <25° = excellent flow; *Carr's index* <20% = fair to excellent flow; *Hausner's ratio* < 1.2 = fair to excellent flow



Figure 2: SEM of (A) Formulation M1 (microsphere containing alginate alone) (B) Formulation M5 (microsphere containing keratin + alginate in the core) (C) Formulation M9 (microsphere containing keratin in the gelling ion) Mag. 400 x

Morphological properties of keratin/alginate microspheres

The result of the microspheres morphology is shown in Figure 2.

In vitro dissolution properties

The *in vitro* release profile of various formulations is shown in Figure 3. The cumulative drug release ranged from 62 - 92 % at 12 h. M1 had 62 % at 12 hours, while M2, M3, M4, and M5 showed a cumulative release of 73 - 95 % at 12 h, but M6, M7, M8, and M9 72 - 92 % at 12 h.

The result of release data fitted into different kinetic models is shown in Table 5. The release kinetics showed the highest linearity ($r^2 = 1.0000$).

FTIR spectra

The result of FTIR is shown in Figure 4. The FTIR of microparticle formulation containing an equal concentration of keratin and alginate reveals the presence of NH symmetric stretching of the 1° and 2° amine at 1640 and 3473 cm^{-1} . The peak at 2102 cm^{-1} is assigned to COOH carboxylic acid stretching vibration of keratin, gluconic acid of alginate and oxo group in carboxylic acid of dexamethasone. The OH stretching vibration of 1° alcohol in keratin protein, dexamethasone, and sodium alginate is assigned to 3265 cm^{-1} . The wavelengths 723, 779, and 872 cm^{-1} are all assigned to C-Cl of chloro stretching vibration of HCl solvent used in extracting the keratin. The wavelengths 984, 1054, 1118, 1703, and 1878 cm^{-1} are assigned to oxo group in the ester group of dexamethasone while 2344, and 2378 cm^{-1} oxo group in the carbonyl of dexamethasone.

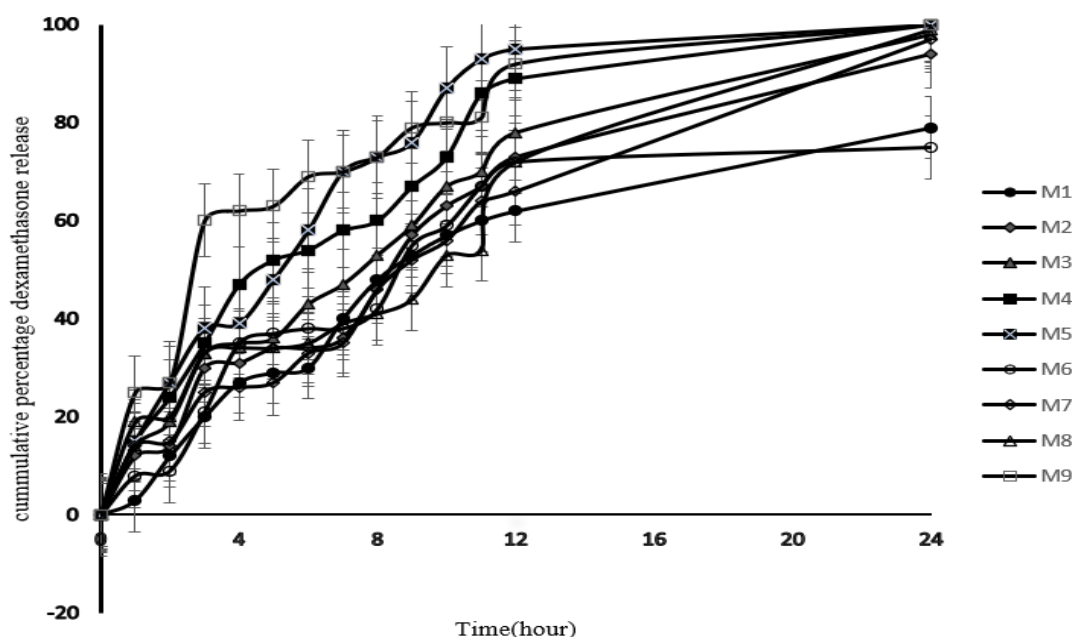


Figure 3: Dexamethasone release from microsphere formulations

Table 5: R^2 Values of the formulations for different release models

Code	Zero-order	First order	Higuchi	Korsmeyer and Peppas	(n)
M1	0.8548	0.9590	0.9429	0.9216	1.009
M2	0.8951	0.9705	0.9421	0.9488	0.7119
M3	0.9028	0.9253	0.9674	0.9796	0.68
M4	0.7918	1.0000	0.9421	0.9117	0.477
M5	0.7443	0.9450	0.8941	0.9186	0.7192
M6	0.7624	0.8179	0.8984	0.8768	0.7958
M7	0.9464	0.9224	0.944	0.9492	0.6796
M8	0.9065	0.8137	0.877	0.7446	0.3772
M9	0.6042	0.8501	0.8579	0.8247	0.4531

Note: $n \leq 0.45$ means Fickian diffusion; $n = 0.45 - 1$ means a non-Fickian or an anomalous diffusion mechanism; $n=1$ means zero order release; $n > 1$ = super case II transport

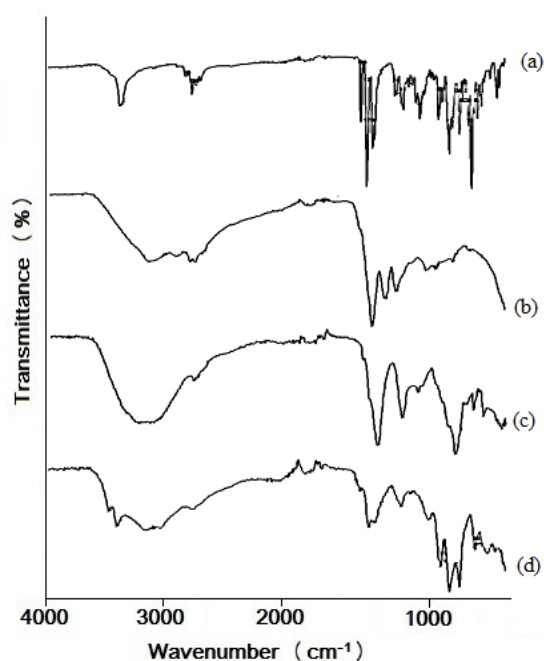


Figure 4: FTIR spectra of (a) Dexamethasone powder, (b) Keratin powder, (c) Formulation M1 (microsphere containing alginate alone), (d) Formulation M5 (microsphere containing high keratin + high alginate)

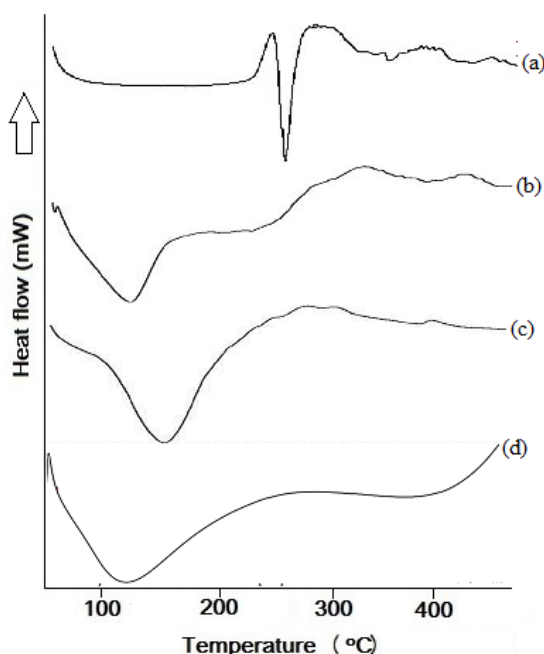


Figure 5: DSC spectra of (a) Dexamethasone powder, (b) Keratin powder, (c) Formulation M1 (microsphere containing alginate alone), (d) Formulation M5 (microsphere containing high keratin + high alginate)

DSC thermograms

The DSC thermogram of keratin shown in Figure 5 revealed a sharp endothermic trough from

280°C for dexamethasone, an endothermic trough at 110 °C, and exothermic peak at 350°C for keratin, formulation 1 showed a sharp peak at 120 °C while microsphere containing equal proportion of keratin and high alginate revealed a broad endothermic peak from 100 to 110 °C.

Stability studies

The result of percentage entrapment efficiency over 3 months is shown in Table 6. There was no significant difference ($p < 0.05$) in the stability of dexamethasone microspheres from various batches.

Table 6: Stability of keratin/alginate microspheres at ambient temperature and humidity

Code	Initial (%)	month 1 (%)	Month 2 (%)	Month 3 (%)
M1	86	86	86	85
M2	91	91	91	90
M3	94	94	94	94
M4	98	98	98	97
M5	98	98	98	97
M6	88	88	88	88
M7	93	93	93	92
M8	97	97	97	97
M9	98	98	98	97

DISCUSSION

Micromeritic is the study of small particles. Bulkiness is important during the packaging of microspheres, and it is determined by the densities of the product, therefore, the packing property of the microspheres was studied using the bulk and tapped densities [11]. The observed densities revealed that there is no significant difference ($p < 0.05$) between the bulk and tapped density. This lack of variation in densities is due to the microspheres having a narrow size range and uniform particle size hence they have low consolidation and high void or interparticle space between them. Flow and compressibility index of the microspheres were studied using the angle of repose (ϕ), Carr's index, and Hausner's ratio [12].

The MS flow showed an angle of repose $< 20^\circ$, indicating an excellent flow. This is supported by Carr's index, and Hausner's ratio values indicating excellent flow of microspheres. Particle size, shape, and density affect flow properties. Generally, spherical particles have better flow than irregular particles [11]. The microsphere flow property observed was due to spherical particle shape, their micro range particle size and density, which shows a consolidated microsphere. The SEM revealed discrete particles. M1 formulation showed particles having

smooth surfaces while microspheres containing keratin revealed discrete particles with very rough surfaces which is attributed to keratin.

Microspheres exhibited a high drug content encapsulated by the polymer composite. The observed high drug entrapment is due to the fast gelation reaction between the alginate and calcium chloride, which caused more drugs to be entrapped in the microsphere matrix [10]. The drug loading shows the weight of dexamethasone contained in each weight of the microsphere which indicates that the weight of dexamethasone in the microsphere was between 11 to 23 %. Drug loading signifies the drug content loaded per unit weight of the microsphere and there was a significant difference in their drug loading ($p < 0.05$).

In vitro dissolution is an important parameter in formulation. Across the batches of microspheres, the polymer composite exhibited retarded drug release in the acid phase followed by a continuous release in phosphate buffer. In microspheres containing keratin, cumulative drug release from M1 to M9 in acid pH was less than 30 % after 2 h. This low drug release was attributed to the adhesive nature of alginate in holding particles together as well as the acid-insoluble nature of keratin. Thus, the release profiles exhibited by the microsphere indicate that the dissolution is pH-dependent and keratin is a pH-sensitive polymer. In M2 – M5, release following the pattern M2 > M3 > M4 > M5.

These similarities and release patterns are attributed to the polymer type and concentration. The drawback of alginate is that it dissolves in the stomach, it is hydrophilic and when exposed in the upper GIT, it swells and releases dexamethasone prematurely while keratin is a biodegradable, non-adhesive polymer. It does not dissolve in upper GIT or acid pH rather it breaks down at a higher pH [13] and aids in drug release, especially in the colon. It was observed that increasing the quantity of keratin and decreasing alginate led to interference with the adhesive ability of alginate thus reducing the polymer's binding ability hence increasing the dexamethasone release. While formulations with a low amount of keratin gave a more sustained drug release.

Polymers are used as drug carriers in microspheres and are used to control the rate of drug release [14]. Since drugs are released from polymer matrices, polymers should have appropriate solubilities. Keratin is insoluble in acid pH but its solubility increases at higher pH. The solubility of keratin in the formulation has a direct effect on dexamethasone release.

Typically, the polymer has to go into the solution first before the drug comes out from it, however, dexamethasone is highly soluble in the aqueous environment. It was observed that an increase in keratin concentration leads to more particles being solubilized in simulated colon pH.

In M6 to M9, there was less dexamethasone release in the acid phase, followed by a continuous release in the alkaline phase that was sustained for 24 hours. These microspheres containing keratin in the core and gelling phases showed that dexamethasone released from M6 and M7 are similar to that obtained from M1 microspheres while M8 – M9 were significantly different from M1 microspheres ($p < 0.05$). Sustained release followed the order M6 > M7 > M8 > M9. Microspheres with the lowest amount of keratin concentration M6 gave more sustained drug release than those containing the highest amount of keratin (M9). In comparison, the reason M2 - M5 varies from M6 – M9 is because M6 – M9 had keratin in the calcium chloride solution which makes them more resistant to dexamethasone release in the acid phase. More so, keratin could form a coat around the microsphere that delays the microsphere from dissolving in the acid phase.

The FTIR analysis reveals changes in the spectra of microsphere formulation due to the presence of different functional groups in keratin, alginate and dexamethasone. It reveals the presence of functional groups such as amino ($-NH_2$) and carboxylic acid ($-COOH$) in keratin and gluconic acid in alginate. It also showed the presence of the hydroxyl ($-OH$) group in keratin, dexamethasone, sodium alginate, and the oxo group in dexamethasone. Therefore, the FTIR spectra of the microsphere expressed spectra patterns that indicate the functional groups present in drug and polymers without additional spectra. The DSC thermogram of the various microspheres containing individual excipients (keratin, alginate) showed no changes in the thermal properties of each constituent in the microsphere when compared with the thermograms of dexamethasone.

Drug release kinetic is the mathematical expression of the drug release rate from a microsphere or microcapsule formulation. The release kinetics reveal that drug release from microspheres is best explained by First-order equation, which showed the highest linearity ($r^2 = 1.0000$). First-order release is the release of a fraction amount of drug from the formulations into the dissolution fluid and this release is dependent on the concentration. Furthermore, drug release from a first-order model is limited to dissolution

and is seen in formulations such as sustained release [15].

CONCLUSION

Dexamethasone-loaded microspheres using keratin and alginate composite have been successfully developed and characterized. This study has demonstrated the potential keratin extracted from chicken feathers as a pH-dependent polymer to develop microspheres for colon-targeted dexamethasone delivery. Further studies in animal models would be required to determine *in vivo* properties of this formulation.

DECLARATIONS

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Ethical approval

Not applicable.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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