A METHOD TO DETERMINE ADHESION OF SUPPOSITORY MASS ON EXCISED INTESTINAL TISSUE

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ABSTRACT

A method to determine adhesion of suppository mass to intestinal tissue was developed using excised pig intestine. The method which employs the principle of drainage unto and subsequent detachment from the mucosa, of an adherent suppository mass is simple, inexpensive and accurate. Fully optimised, it can be used to assess differences between suppository formulations, as shown by the preliminary results obtained with commercial Anusol formulations.

Keywords: Adhesion, Suppository mass, Excised intestinal tissue, Perfusion, Detachment

INTRODUCTION

The potential use of the rectum for the systemic delivery of drugs is a relatively recent idea, even though administration of drugs in the rectum, using the suppository dosage form, is an old practice (Lieberman and Anschel, 1979; Helliwel, 1993; Adikwu and Okafor, 2006).

The advantage of rectal delivery must be the reduced extent of hepatic first pass elimination of drug, especially when the drug is administered in the lower region of the rectum (Onyechi and Martin, 1995).

The limited results obtained with oral bioadhesive systems for enteral delivery drive the search for formulations for rectal delivery. Such systems should prolong the gastrointestinal (GIT) transit time of the drug delivery system and subsequently improve the bioavailability of drug loaded into it (Kellaway *et al*., 1984; Pritchard *et al.*, 1996; Helliwel. 1993; Smart *et al*., 1994; Leung and Robinson, 1990; Lehr *et al.,* 1990).

To date drainage and perfusion techniques are used in bioadhesion studies. These techniques provide some approximation to *in vivo* conditions (Kellaway et al, 1984; Pritchard *et al*, 1996; Helliwel, 1993; Smart *et al*, 1994; Onyechi and Martin, 1995; Leung and Robinson, 1990; Lehr *et al.,* 1990).

The aim of this study was to design intestinal drainage and detachment techniques as well as model drainage and detachment of commercial Anusol products. Such techniques developed would then be applied to the development of sustained release (SR) bioadhesive formulations for Anusol products.

MATERIALS AND METHODS

Animal Model: Intestinal tissue was obtained from freshly slaughtered pits. After cleaning and bathing in normal saline, the tissue was cut into 10 – 12 cm pieces and stored in self-sealed freezer bags at -16^{0}C until required.

Formulations: Suppository samples formulated at Parke-Davies were used in the drainage studies. For detachment experiments, 0.4 % w/w Fluorescein (BDH Chemicals Limited, Poole, England) was incorporated into the suppositories as marker. Suppositories containing Fluorescein were prepared by melting the supplied suppositories with minimum amount of heat and incorporating the required weight of material. The suppositories after cooling were stored at room temperature.

Experimental Design

***In-Vitro* Drainage Tests:** A metallic stand (Figure 1) was constructed which allowed intestinal tissue to be mounted and maintained vertically in the stand. Once the tissue was mounted, the stand was suspended vertically in a high humidity environment (Figure 2), and allowed to equilibrate in a hot air oven with temperature set at 39 ^{0}C. The suppository under test was weighed and inserted at the upper end of the mounted, excised pig intestinal tissue and allowed to drain. A sample vial (of known weight) was placed beneath the tissue assembly to collect the molten and detached suppository mass. After complete drainage, the sample vial and contents were stored in a desiccator overnight and weighed. Storage in a desiccator enabled removal of moisture adhering to the vial and contents. A fresh piece of intestinal tissue was used for each run (n=4).

***In-Vitro* Detachment Studies:** A water-jacketed glass support was constructed which allowed the mounted tissue to be maintained horizontally (Figure 3). Once the tissue containing the test suppository was mounted, the support was clamped vertically in an environment of high relative humidity and introduced into a hot air oven with temperature set at

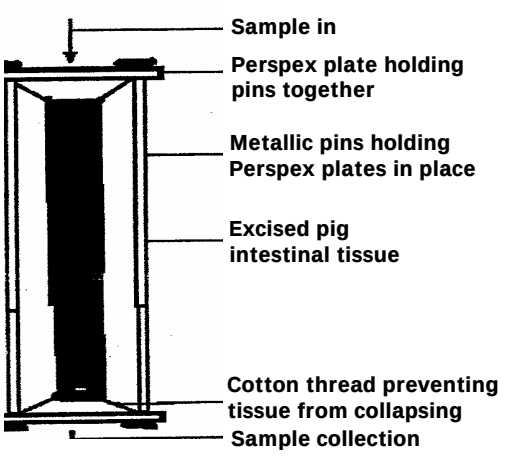

Figure 1: Device for suppository drainage/ adhesion experiments

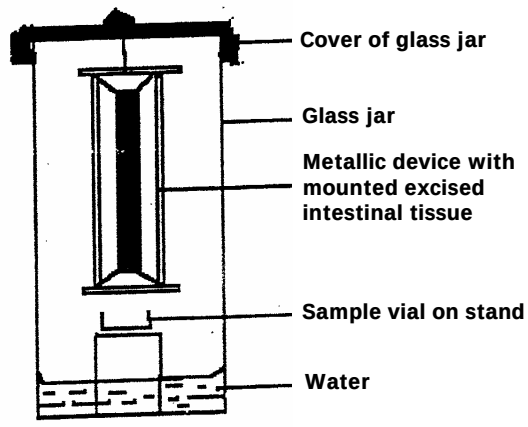

Figure 2: High humidity chamber for drainage experiments

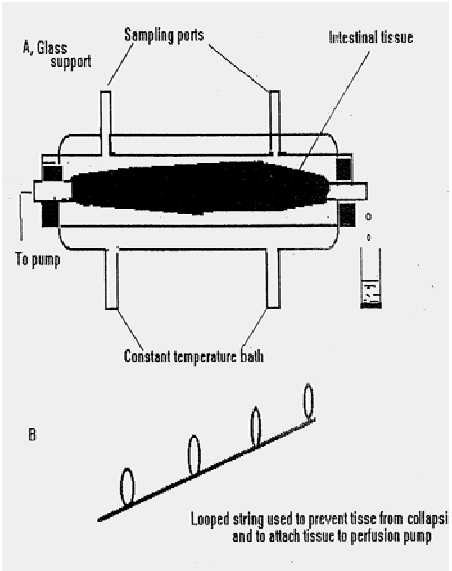

Figure 3: Water-jacketed support for *in-vitro* detachment experiments

39 ^{0}C. The suppository was allowed to drain. Any molten suppository mass which drained down the lumen of the intestinal tissue and broke away was collected and weighed. When drainage ceased, the intestinal tissue was mounted horizontally in the glass support and challenged with a flowing medium, at a fixed rate of 4 ml/minute. Distilled water maintained at 39 ^{0}C was perfused through the intestine with the aid of peristaltic pump. Perfusate samples were collected over a period of 3 hours. Sampling jars were replaced at 30 minutes intervals. A fresh tissue was used for each run (n = 3). The contents of the vial were assayed for fluorescein by spectrophotofluorimetry.

Analytical Method for the Determination of Fluorescein: Analytical techniques employed for the quantitative determination for drugs in delivery systems play a significant role in the evaluation and interpretation of data in adhesion studies. It is essential to employ well-characterised and validated analytical methods to yield reliable results which can be satisfactorily interpreted. The appropriateness of a technique is influenced the ultimate objective of the study. The size of sample involved in the detachment studies and the need for specificity, speed and economy influenced the choice of fluorimetry in the work.

The determinations were performed with a Perkin Elmer Spectrophotofluorimeter Model MDF-2A (Perkin Elmer, Norwalk, Connecticut, USA). Standard solutions of fluorescein in 0.001 M NaOH were prepared ranging from 0.05 to 0.2 ppm. The solutions were assayed at 513 nm; the wavelength of maximum emission for fluorescein and in 0.001 M NaOH is 495 nm. The calibration curve of emission intensity against concentration fluorescein constructed at this wavelength is shown in Figure 4.

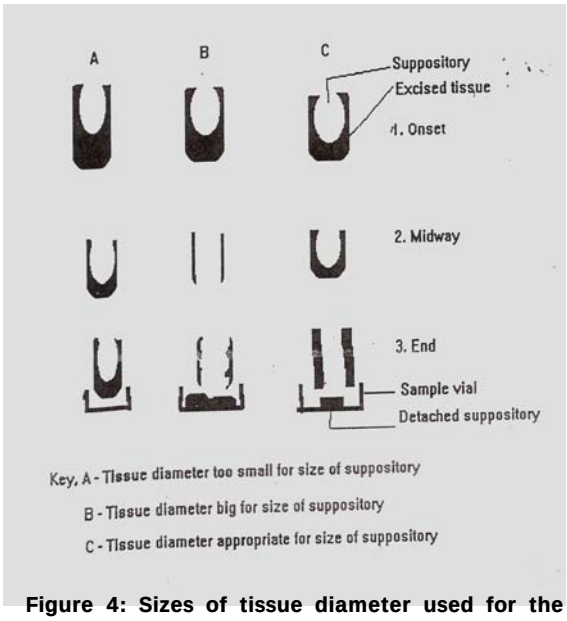

Figure 4: Sizes of tissue diameter used for the suppository

Determination of the Fluorescein Content of Anusol Suppositories: The Anusol suppositories prepared for the drainage experiments were assayed for fluorescein content. A limitation imposed by the number of suppositories prepared led to the use of only a single suppository for the test.

Aliquots of this single suppository were used. An accurately weighed amount of the suppository (5 mg) was transferred to a 500 ml volumetric flask. 25 ml of 0.001 M NaOH was added and warmed to 80 ^{0}C for 2 minutes in an ultrasonic water bath. The solution was cooled and made up to volume with 0.001 m NaOH. The resultant solution was filtered and assayed for fluorescein content. The determination was repeated 5 times.

Validation of Assay Technique: The recovery of fluorescein from the suppository mass was evaluated in order to validate the method of assay. An accurately weighed sample of the suppository mass containing fluorescein was transferred into a 200 ml volumetric flask. 25 ml of 0.001 M NaOH was added and warmed to 80 ^{0}C for 2 minutes in an ultrasonic water bath to dissolve the suppository and content. 0.001 M NaOH solutions were used to make up to volume. After filtration the solution was assayed for fluorescein content. The mass of suppository used for the validation of the assay technique was varied between 2 and 20 mg. The experiments were repeated three times for each sample size.

Determination of Fluorescein Content of Solution from Detachment Experiments: Perfusate samples were collected at 30 minute intervals from the tissue used for the detachment studies. Each sample was transferred quantitatively into a 500 ml volumetric flask and placed in an ultrasonic water bath and warmed up to 80 ^{0}C for 2 minutes. The solution was cooled and made up to volume with 0.001 M NaOH. After filtration, the samples were assayed for their fluorescein content using the analytical procedure described above. Samples were diluted before assay where necessary using 0.001M NaOH solution. The results of the detachment experiments have been expressed as percent fluorescein detached over the entire 3 hours test period. Total fluorescein detached was determined from the fluorescein content of the perfusate for the 3 hours time. The fluorescein remaining in the excised intestinal tissue at the end of the experiment was not considered in calculating percent fluorescein detached.

RESULTS AND DISCUSSION

The calibration curve of Figure 5 and the assay results of Table 1 showed that the suppositories into which fluorescein were incorporated can be assayed satisfactorily by spectrophotofluorimetry. The recovery of fluorescein using varying sample sizes (Figure 6) indicated that there exists a linear relationship between sample size and fluorescein content.

Drainage Experiments: Considerable effort was spent modifying the metallic stand for the drainage experiments. The original design involved inserting used intestinal tissue around three metallic pins fitted to a wooden base. The pins were used to hold the tissue apart to permit drainage.

Table 1: Fluorescein content of Anusol suppositories

Weight of Suppository Mass taken (mg)	Fluorescein Content (mg)	Unit Fluorescein Content (mg)
4.70	0.01928	0.00410
6.20	0.02518	0.00406
5.90	0.02302	0.00390
8.80	0.03722	0.00423
7.20	0.03027	0.00420

Mean = 0.00420, CV% = 3.17

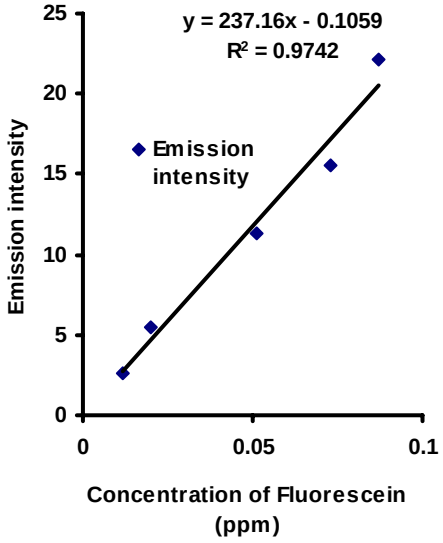

Figure 5: Calibration curve for the determination of fluorescein in 0.001 M NaOH

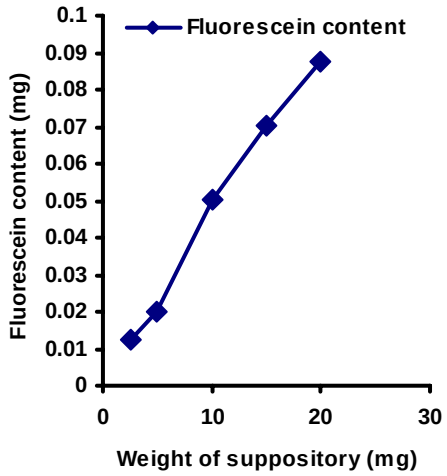

Figure 6: Validation of assay technique for the assay of fluorescein contained in Anusol suppositories

The metallic pins proved not to be stable and the tissue when mounted was difficult to hold in place especially when the suppository was inserted to initiate the experimental procedure. Also, contact between suppository and tissue wall was minimised by the presence of the pins within the gut tissue.

Table 2: Validation of fluorescein assay technique for Anusol suppositories

Weight of Sample	Fluorescein Content (mg)	Unit Fluorescein Content (mg)
2.8	0.01380	0.00492
2.6	0.01126	0.00492
2.4	0.01343	0.00559
5.4	0.02351	0.00435
5.7	0.01733	0.00304
5.4	0.02104	0.00390
11.7	0.04252	0.00363
10.0	0.04103	0.00410
11.7	0.06252	0.00534
15.0	0.06713	0.00448
15.6	0.07207	0.00534
15.5	0.07328	0.00473
21.8	0.08857	0.00406
22.2	0.08633	0.00389
21.3	0.08539	0.00401

Mean = 0.00433, CV% = 15.5

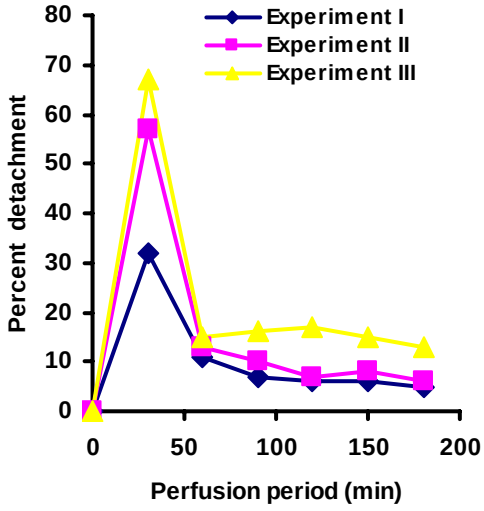

Figure 7: Percentage detachment of Anusol suppositories from gut tissue in in-vitro adhesion studies

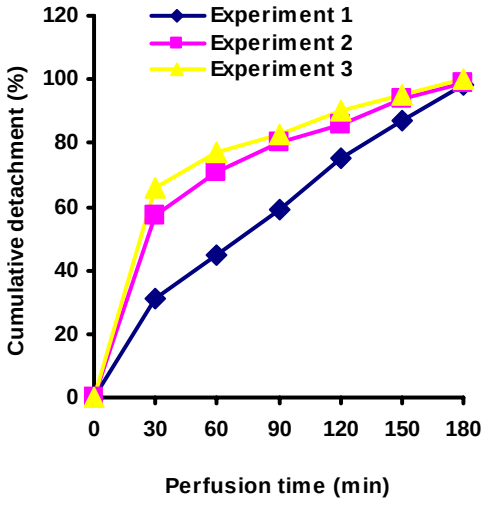

Figure 8: Cumulative percent detachment of Anusol suppositories (batch Number 3 x 126) manufactured on January 19, 1994

There was appreciable delay in the onset of drainage as a result of the positioning of the pins.

In the new improved design, cotton thread was used to prevent the tissue from collapsing. The thread was tied to metallic pins which in no way interfered with the mounting of the tissue. The contact between suppository and gut wall was therefore maximised. The other advantage of using the cotton thread to hold the tissue apart is that it allows a realistic tone on the gut wall to be established.

Results obtained with Anusol GSL suppositories (Batch No. 3 x 126) are shown in Table 2. The time taken for the suppository mass to drain is given results in this manner is that the suppository mass was found to emerge as a bolus from the lower side of the vertically mounted tissue. The time lags between the emergence of the bolus from the tissue tip and its detachment, for the samples of suppositories examined, varied between 30 and 120 sec. It was therefore decided to record the time between the start of the experiment and that for the hanging bolus of molten suppository to detach, as the drainage time.

A considerable length of time was spent identifying factors which affect the drainage experiments. The factors can be separated into tissue, suppository, test device and test environment related factors.

The internal diameter of tissue relative to the size of suppository being tested was the single most important factor. Rat and rabbit intestinal tissue were used initially to establish the technique. In the situation where the size of suppository was too large relative to that of the tissue, there was considerably delayed onset of drainage which eventually ceased despite the presence of molten suppository mass. Where the size of suppository was too small, the coating to the intestinal wall was patchy and drainage time too fast. With an optimum size of tissue, the suppository coated the intestinal wall uniformly and copiously. A diagrammatic representation of what was observed is shown in Figures 7 and 8. It is reasonable to suggest that there is an optimum tissue to suppository size which will yield acceptable and reproducible coating of the excised intestinal wall lumen.

Samples of Anusol FSL suppositories were used for repeat drainage experiments. The experiment was aborted after 5 hour of test at 39 ^{0}C. The excised intestinal tissue with the suppository was left on the shelf to dry out and cut open vertically. The suppository appeared molten, had distended the excised tissue but could not flow or drain out. The central portion of the suppository had started coating the tissue but was not fluid enough at the test temperature, to drain unaided.

The influence of the test device is related to its length. The length of tissue and therefore the time taken for any enclosed, molten suppository mass to drain out will depend on the length of the device. The device used in this study accommodates 9 cm of excised intestinal tissue.

Table 3: Drainage experiments with Anusol GSL suppositories (Batch No 3x126)

Sample Number	1	2	3	4
Wt of sample (g)	2.67410	2.65349	2.65865	2.71971
Weight of sample drained out (g)	1.23161	0.98631	1.12381	1.35421
% Suppository retained on tissue	53.94	62.83	57.73	50.21
Mean % retained	56.18 (9.60)*			
Time taken for drainage (min)	50	50	45	69
Mean drainage time (min)	53.50 (19.81)*			

**CV%*

Table 4: Detachment of Anusol suppositories (Batch Number 3 x 126)

Sampling time (min)	I		II		III		Mean	
	Detachment							
	mg	%	mg	%	mg	%	mg	%
30	0.21	57.02	0.25	66.39	0.09	31.19	51.53	(35.38)*
60	0.05	13.95	0.05	12.20	0.04	15.38	13.84	(11.50)
90	0.04	9.96	0.02	6.20	0.04	14.54	10.1	(39.33
120	0.02	6.37	0.02	5.60	0.04	14.54	8.83	(56.04)
150	0.03	7.95	0.02	5.60	0.04	13.75	9.10	(46.10)
180	0.02	4.75	0.02	4.00	0.03	11.72	6.82	(62.42)

Table 5: Cumulative Percent Detachment of Anusol Suppositories (Batch Number 3 x126)

Sampling time (min)	I	II	III	Mean Cumulative Detachment
30	57.0	66.39	31.19	51.35 (35.38)*
60	70.97	78.59	46.57	65.38 (25.58)
90	80.39	84.78	60.71	75.48 (17.13)
120	87.30	90.39	75.25	84.31 (9.49)
150	95.25	95.99	89.00	93.41 (4.11)
180	100	100	100	100

** CV%*

It was observed that when the drainage experiment was performed in an oven, there was drying out of intestinal tissue as the experiment progressed. There was the need to perform the experiment in a high humidity environment. A glass jar containing some water (or normal saline) was used to maintain a high humidity environment (see Figure 2). This step was necessary because in the body, tissues and organs are bathed in physiological fluids.

Other factors which were identified which may have effects on the reproducibility of test results were: (i) use of fresh or thawed, previously-frozen, intestinal tissue samples (ii) treatment of intestinal tissue before drainage or detachment experiments and (iii) use of the small or large intestines. Fresh samples were found to be coated more efficiently than stored samples. Draining suppository masses tend to egress with some intestinal tissue attached to them when the mounted intestinal tissue had been previously stored at -20 ^{0}C. The influence of the portion of intestine used is thought to be related to the internal diameter of the excised tissue compared to that of the suppository. Small intestinal tissue portions have a smaller internal diameter and therefore promote better coating and adhesion of the melted suppository to the tissue mucosa, as discussed above.

Perfusion / Detachment Experiments: The suppository mass was allowed to adhere to the pig intestine prior to the perfusion apartments. A looped string made of stainless steel was used to support the intestinal (Figure 3) and prevent it from collapsing while allowing the inserted suppository sample to drain (Figure 2). The molten suppository mass drained out of period of time, the time for this drainage being dependent on the diameter of the supporting wire (Table 3). It was necessary to optimise the size of this metallic support, to ensure full coating of the lumen of the excised intestinal tissue. The perfusion experiments were started only after complete drainage of suppository mass and coating of intestinal tissue had occurred.

The choice of a 4ml/minute flow rate for the perfusion experiments was arbitrary. It was planned to vary the perfusion rate and the 4ml/minute flow rate was a convenient starting point. The extent of adhesion to gut tissue was determined by calculating percent detachment during the period of perfusion. This was done over a 3 h period. In Table 4, the percent detachments for the suppositories prepared. The data are represented graphically in Figure 7. The cumulative percent detachment for the same samples over a 3 hour period is shown in Table 5. This is depicted graphically in Figure 8. The data in the tables and figures showed that there was considerable adhesion to the intestinal mucosa. Within the first 30 minutes of the experiment, loose and detached suppository mass which formed during the draining of excess suppository mass, seen to egress rapidly in the perfusate. This occurred within the first 5 minutes. Thereafter, there was gradual release of suppository mass into the perfusate. The preliminary result suggests that with further optimisation of the technique, reproducible results can be obtained.

Conclusion: The practical basis for the use of excised pig intestinal tissue for adhesion experiments has been established.

A device which can be used to mimic *in vitro* what happens to the suppository *in vivo*, was developed. Preliminary data obtained with the device showed that the results were reproducible and accurate. The factors that may influence results obtained with this device were identified and relate to the type of formulation being investigated, type of test tissue, test device and the test environment. Perfusion models to confirm the results of the detachment experiments were established. The various factors that may influence results obtained with this model are currently being optimised. It is expected that the test system developed will be used in satisfactory assessment of formulation variables of the commercial Anusol products.

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REFERENCES

ADIKWU, M. U. and OKAFOR, J. O. (2006) Evaluation of mucus as a release enhancer for rectal delivery of glibenclamide. *Current Drug Delivery,* 3(2): 1 – 12.

HELLIWEL, M. (1993). The use of bioadhesion in targeted delivery within the gastrointestinal tract. *Drug Delivery Review*, 11: 221 – 251.

HELLIWEL, M. and SEKTERE, M. A. O. (1993). The formulation of bioadhesive suppositories. Pages: 79 – 100. *In:* URTTI, A. (Ed). *Methods to overcome biological barriers in drug delivery,* Kuopio University Publications, Japan.

LEHR, C.M., BOUWSTRA, J. A., SCHACHT, E. H. and JUNGINGER, H. E. (1990). Pages 123 – 145. *In:* LEHR, C. M. (Ed). *Bioadhesive drug delivery systems for oral application*, Leiden University, The Netherlands.

LEUNG, S. H. S. and ROBINSON, J. R. (1990). Bioadhesives in drug delivery. *Polymer News,* 15: 333 – 342.

LIEBERMAN, H. A. and ANSCHEL, J. (1979). Suppositories. Pages 538 – 560. In: LACHMAN, L., LIEBERMAN, H. A. and KANIG, J. L. (Eds.). *The Theory and Practice of Industrial Pharmacy.* Lea and Febiger, Philadelphia.

PRITCHARD, K. LANSLEY, A. B., MARTIN, G. P., HELLIWELL, M. MARRIOTT, C. and BENEDETTI, L. M. (1996). Evaluation of the bioadhesive properties of hyaluronan derivatives: detachment weight and mucocilliary transport rate studies. *International Journal of Pharmaceutics,* 129: 137 – 145.

ONYECHI, J. O. and MARTIN, G. P. (1995). Adhesion studies on Anusol Formulations: Summary report. A *Technical Report for Parke Davies and Company, Limited, Usk Road, Pontypool, Gwent, United Kingdom.*

OFOKANSI, K. C. (2004). *Evaluation of mucin-gelatin mucoadhesive microspheres for rectal delivery of some cephalosporins*. PhD Thesis, University of Nigeria, Nsukka.

SMART, J. D., KELLAWAY, I. W. and WORTHINGTON, E. C. (1984). An in vitro investigation of mucoadhesive materials for use in controlled drug delivery. *Journal of Pharmacy and Pharmacology,* 32: 295 – 299.