Research paper

Polymer and microsphere blending to alter the release of a peptide from PLGA microspheres

Harish B. Ravivarapu[a], Kevin Burton[b], Patrick P. DeLuca[c,*]

[a]Dynavax Technologies Corporation, Berkley, CA, USA
[b]Purdue Pharma L.P., Ardsley, NY, USA
[c]Faculty of Pharmaceutical Sciences, University of Kentucky, College of Pharmacy, Lexington, KY, USA

Abstract

The objective of this study was to evaluate the effect of polymer and microsphere blending in achieving both a sufficient initial release and a desired continuous release of a peptide from poly(D,L-lactide-co-glycolide) microspheres. Leuprolide acetate loaded hydrophilic 50:50 PLGA microspheres were prepared by a solvent-extraction/evaporation process and were characterized for their drug load, bulk density, size distribution, surface area, surface morphology, in vitro drug release, and in vivo efficacy. Combining PLGA polymers that varied in their molecular weights in various ratios yielded microspheres with varied drug release profiles commensurate with the hydration tendencies of the polymers. Increasing the component of lower molecular weight 50:50 hydrophilic PLGA polymer, 8.6 kDa increased the initial drug release. A similar microsphere formulation prepared instead with blending microspheres from individual polymers showed a similar increase. In an animal model, microspheres obtained from polymer or microsphere blends attained a faster onset of testosterone suppression as compared to microspheres from higher molecular weight 50:50 hydrophilic PLGA polymer, 28.3 kDa, alone. These studies illustrated the feasibility of blending polymers or microspheres of varied characteristics in achieving modified drug release. In particular the increased initial release of the peptide could help avoid the therapeutic lag phase usually observed with microencapsulated macromolecules. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: PLGA microspheres; Leuprolide, Peptide release; Polymer and microsphere blend

1. Introduction

Compared with conventional low molecular weight molecules, proteins and peptides have unique requirements and limitations for delivery. These agents in general have short plasma half-lives, are unstable in the gastrointestinal (GI) tract, and also have low bioavailabilities due to their large molecular weights and high aqueous solubility [1,2]. Frequent injections might be acceptable in cases of acute situations but not in chronic conditions. Thus, development of sustained release dosage forms for peptide/protein delivery will help these agents to realize their full potential as drugs while enhancing patient compliance and convenience.

Microspheres prepared from poly(D,L-lactic-co-glycolic) acid (PLGA) polymers have been studied extensively in the last two decades as sustained release dosage forms and have shown improved patient compliance and/or therapeutic efficacy of contraceptive steroids, narcotic antagonists, antimalarials, and anticancer agents [3,4]. Recent studies, especially with luteinizing hormone-releasing hormone (LHRH) analogues, have shown these systems to be effective in the sustained delivery of macromolecules [5–7]. In addition to being biocompatable, degradation rates of PLGA and the accompanying release of encapsulated drug can be controlled by the polymer's physical properties such as molecular weight, hydrophilicity, and the ratio of lactide to glycolide [8–11]. Thus, it is possible to obtain the desired drug release from PLGA microspheres by altering the polymer's characteristics. An extension to this approach is to optimally combine microspheres prepared from different polymers with known drug release or to blend polymers prior to preparing the microspheres. The effect of mixed populations of controlled release particles on the resulting release pattern has been cited in some early literature reports [12–14] but there has been little experimental follow-up to show the feasibility and practical application of blending.

The purpose of this study was to prepare and evaluate

* Corresponding author. University of Kentucky, College of Pharmacy, Rose Street, Rm 327G, Lexington, KY 40536-0082, USA. Tel.: +1-606-257-1831; fax: +1-606-323-0242.
E-mail addresses: Hravivarapu@dvax.com (H.B. Ravivarapu), kevin.burton@pharma.com (K. Burton), ppdelu1@pop.uky.edu (P.P. DeLuca).

peptide-loaded microspheres from various blends of two commercial PLGA polymers with different molecular weights as an alternative to modifying the polymer characteristics in achieving enhanced initial drug release. Further, formulations obtained by physically blending microspheres that were prepared from individual polymers were evaluated as an alternative approach to making microspheres from blended polymers. LHRH superagonist analogue leuprolide acetate was selected and peptide-loaded microspheres from hydrophilic PLGA (50:50) polymers were prepared by a solvent extraction/evaporation method. Physico-chemical characteristics of the microspheres were correlated with the in vitro peptide release, and the formulation efficiency in suppressing serum testosterone levels for sustained periods was evaluated in an animal model.

2. Materials and methods

2.1. Materials

PLGA (50:50) polymers, Resomer® RG503H (MW 28, 032) and RG502H (MW 8631) were obtained from Boehringer Ingelheim (Ingelheim, Germany). LHRH analogue, leuprolide as an acetate salt was purchased from Bachem Inc. (Torrance, CA). All other chemicals used were of analytical reagent grade.

2.2. Preparation of microspheres

Microsphere formulations in a typical batch size of 1.5 g were prepared by a solvent-extraction/evaporation method [15]. PLGA polymer or a combination of PLGA polymers in methylene chloride was mixed with a methanolic solution of the peptide. The resulting mixture (dispersed phase, DP) was added to a 0.35% w/w polyvinylalcohol (PVA, MW 30–70 kDa, Sigma) aqueous solution (continuous phase, CP) while stirring at 7000 rev./min using a homogenizer (Silverson L4R, Silverson Instruments Corp., MA). After 5 min at 25°C the stirring rate was decreased to 500 rev./min and the temperature raised to 40°C to slowly extract and evaporate the organic phase over 1 h. After cooling to 25°C, particles were recovered by filtration and dried overnight under vacuum at room temperature.

To evaluate the effect of polymer blends, the 8.6 kDa polymer was combined in various proportions with

28.3 kDa polymer as detailed in Table 1 (formulations C–E). Total polymer concentration was adjusted so that the viscosities of polymer/methylene chloride solutions, as measured by a Brooksfield viscometer, were comparable. The target drug loading for all the formulations was 12.5% w/w. Microspheres prepared from individual polymers were mixed physically in 3:1 (28.3 kDa/8.6 kDa) drug content ratio to obtain formulation F. Characteristics of these microspheres were compared with those of microspheres prepared from a 3:1 polymer mixture (formulation C).

2.3. Characterization of microspheres

The microspheres were characterized for drug content, bulk density, specific surface area, mean particle size, surface morphology, and in vitro drug release and in vivo efficacy.

2.3.1. Drug content

Drug loaded microspheres were quantitatively dissolved in methylene chloride and the peptide was extracted into acetate buffer (pH 4, 0.1 M) by shaking the mixture for 1 h on a wrist action shaker (Burrell, Pittsburgh, PA). The aqueous buffer phase was separated by centrifugation and extracted peptide was quantitated by a reverse phase-HPLC method [16] after some modifications. The extraction was repeated with fresh buffer and the combined peptide amount values were reported as the drug content, and expressed as % w/w of microspheres. Triplicate samples were used for determining the drug content and mean values were reported. HPLC analytical conditions were as follows: chromatograph separation was achieved on a C_{18} μBondapak column (3.9 × 300 mm, Waters) using a variable wavelength detector at 220 nm, a gradient pump (both from Dionex Corp., Sunnyvale, CA) and an autosampler (Thermo Separation Products, Fremont, CA). The mobile phase was a 68:32 isocratic mixture of HPLC grade water and acetonitrile, which was adjusted to pH 4.0 with 0.1% trifluoracetic acid. The flow rate was 1.1 ml/min.

2.3.2. Bulk density

The dry microspheres were quantitatively transferred to a graduated test tube. The test tube was subsequently tapped

Table 1
Manufacturing parameters of peptide-loaded microspheres[a]

Formulation ID	A	B	C	D	E
Polymer(s)	28.3 kDa	8.6 kDa	28.3 kDa/8.6 kDa	28.3 kDa/8.6 kDa	28.3 kDa/8.6 kDa
Ratio			3:1	4:1	5:1
w/w % of polymer in DP[b]	16.3	38.0	24.0	21.1	19.6
Ratio of CH_3OH/CH_2Cl_2 in DP	0.24	0.22	0.20	0.20	0.20

[a] Formulation F was a physical 3:1 combination of A and B.
[b] DP, dispersed phase containing peptide and polymer in methanol and methylene chloride.

20 times from a vertical distance of approximately 0.5 inches and the occupied volume recorded. The tapping process was repeated until the volume occupied by particles remained unchanged. The final volume was recorded as bulk volume, v_b, and the tapped bulk density (g/cc) was calculated as m/v_b, where m was the weight of microspheres employed.

2.3.3. Specific surface area

The specific surface area was determined using an ASAP 2000 surface area analyzer (Micromeritics, Norcross, GA) by BET transformation of the adsorption-desorption isotherms of Kr on the surface of microspheres. The area values were normalized to the sample weight, which was typically in the range of 250–300 mg.

2.3.4. Size distribution

Particle size distribution was determined using a Malvern 2600c Laser Diffraction Particle Sizer (Malvern Instruments, Southborough, MA). The microspheres were suspended in pre-filtered 0.1% aqueous Tween 80 solution and either a 63 mm (for a size range of 0.5–118 μm) or 100 mm (for a size range of 1.9–188 μm) focal length lens was employed to determine particle size. Mean diameter based on volume was determined.

2.3.5. Surface morphology

The surface morphology was examined by scanning electron microscopy (Hitachi Model S800, Japan) after coating the microsphere sample with gold-palladium on an aluminum stub.

2.3.6. In vitro drug release

Approximately 10 mg of peptide loaded microspheres were quantitatively transferred to test tubes and incubated with 10 ml of 0.033 M phosphate buffer (pH 7) at 37°C in a temperature controlled oven. Separate samples were maintained for each time point. The tubes were shaken twice weekly and 8 ml of supernatant were replaced with fresh buffer every 7 days to maintain sink conditions. After sampling, microspheres were separated by centrifugation. To minimize the loss of microspheres, only 80% of the supernatant was removed. Correction for peptide in the remaining 2 ml of supernatant was made in the final calcu-

lations of peptide remaining in microspheres. The drug content in the microspheres was quantitated as described earlier by HPLC.

Peptide release was based on the peptide remaining in the microspheres rather than on the released amount of peptide, as the released peptide has limited stability (unpublished laboratory studies) in the in vitro releasing medium under the experimental conditions. Released drug was calculated as the difference between initially loaded drug and that remaining in the microspheres, and expressed as a % of initially loaded amount.

2.3.7. In vivo evaluation

Male Sprague–Dawley rats (Harlan Sprague Dawley, Inc., Chicago, IL) at least 12 weeks old, weighing 200–250 g were employed ($n = 6$ per formulation) to assess serum testosterone levels. Animals were maintained as per the guidelines set forth in Guide for the Care and Use of Laboratory Animals, DHEW Pub. No. (NIH) 78-23 (revised). The microspheres were suspended in a mixture of 1% carboxymethylcellulose (7LFPH, USP, Aqualon, Delaware, NJ) and 2% mannitol (USP/EP) and injected into rats subcutaneously just below the neck region, at a drug dose of 3 mg leuprolide/kg body weight based on literature reports [17]. A single injection was given to each animal immediately after collecting an initial sample from the tail vein. Further samples were collected at 0.25, 1, 4, 8, 15, 25, 32, 33, 42, and 43 days after dose administration. On days 32 and 42, animals were challenged with 100 μg/kg of leuprolide acetate, to investigate whether the LH receptors were still down-regulated. Following the challenge doses, additional samples were taken at 6 and 24 h. The lack of an elevation in testosterone levels above 0.5 ng/ml would indicate that the receptors were still occupied. Samples were assayed in duplicate for testosterone levels by radioimmunoassay using a standard commercial kit (Active Testosterone®, Diagnostic Systems Laboratories, Webster, TX).

3. Results and discussion

3.1. In vitro characterization

The physico-chemical characteristics of peptide loaded

Table 2
Characteristics of peptide loaded microspheres[a]

Formulation ID (polymer-ratio)	Drug content (%w/w)	Surface area (m²/g)	Size[b] (μm)	Bulk density (g/cc)
A (28.3 kDa)	11.88	0.387	18.0	0.54
B (8.6 kDa)	11.34	1.540	21.0	0.30
C (28.3/8.6 – 3:1)	9.87	0.584	28.0	0.52
D (28.3/8.6 – 4:1)	9.48	0.420	28.5	0.55
E (28.3/8.6 – 5:1)	9.75	0.602	20.0	0.56

[a] Formulation F was a physical 3:1 combination of A and B.
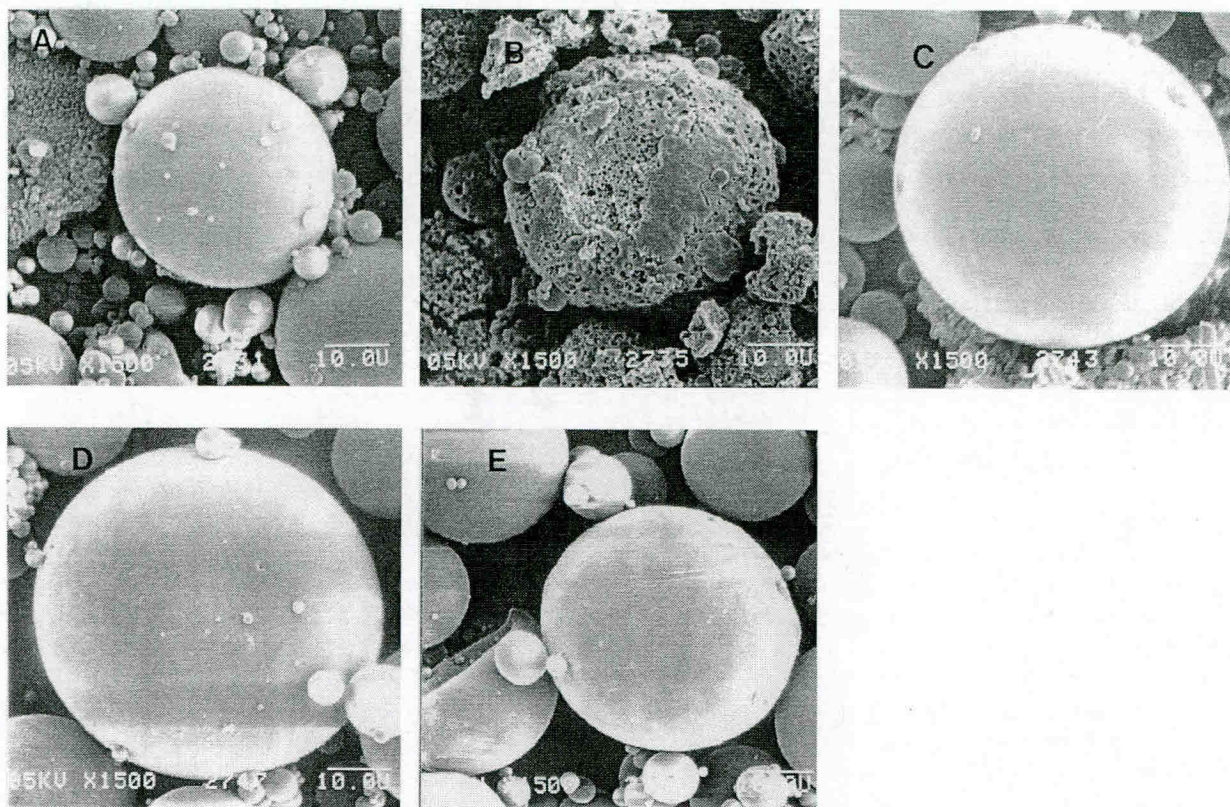[b] Mean diameter based on volume.

Fig. 1. Scanning electron micrographs of microspheres from polymer combinations at 1500× (28.3 kDa/8.6 kDa: A, 1:0; B, 0:1; C, 3:1; D, 4:1; E, 5:1).

PLGA microspheres are tabulated in Table 2 and scanning electron photomicrographs of representative microspheres from each formulation at 1500× magnifications are shown in Fig. 1. All the formulations except for the 8.6 kDa microspheres had a similar surface morphology. In general, microspheres were spherical, smooth, and non-porous. In contrast, the 8.6 kDa microspheres were very porous with rough surfaces and were expected to have high specific surface area and release drug faster.

The formulations had a similar size distribution with mean diameters in the range of 18–29 μm. As the viscosity of polymer solution can influence the microsphere charac-teristics including size distribution, similar polymer viscos-ities in the organic phases were maintained by modifying polymer concentrations (Table 1). In the case of the lower molecular weight polymer, the viscosity was maintained by increasing the polymer concentration. The microspheres used in this study were in an injectable range for convenient subcutaneous and intramuscular injections via a 21- or 23-gauge needle.

Drug content (% w/w) values for 28.6 and 8.6 kDa micro-spheres were similar; however, microspheres from the poly-mer combinations have a decreased loading efficiency (Table 2). The encapsulation efficiency values for all the formulations calculated against the target drug loading of 12.5% w/w ranged from 78 to 95%. Slightly higher amounts

of methylene chloride were used in preparing the 'polymer combination' batches and possibly due to this, higher resi-dual levels of methylene chloride (in the range of 132 vs. <20 ppm with single polymer formulations; data not shown) were observed. It was possible that as methylene chloride was extracted at an apparent slower rate, a higher amount of peptide was lost into the aqueous phase through the polymer wall that remained soft and permeable for a
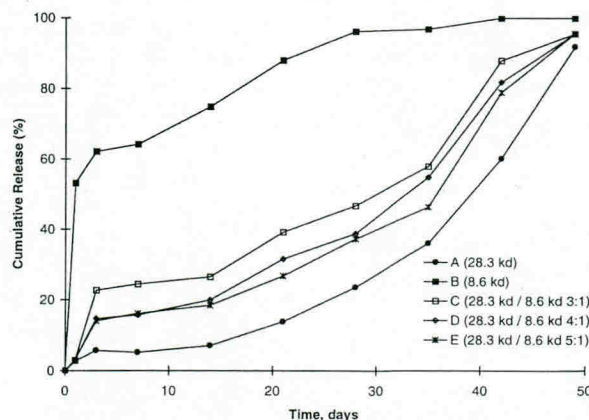

Fig. 2. In vitro peptide release from microspheres prepared from polymer combinations (28.3 kDa/8.6 kDa: A, 1:0; B, 0:1; C, 3:1; D, 4:1; E, 5:1).
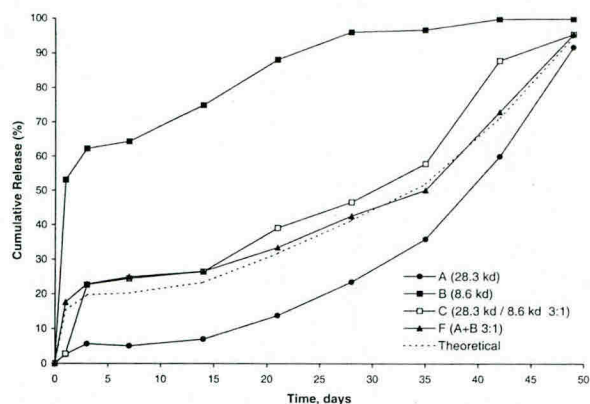
Fig. 3. In vitro peptide release from microspheres prepared from polymer C and microsphere F blends.

relatively longer time in case of combination formulations leading to lower drug content values.

Microspheres prepared from 8.6 kDa polymer in this study had the lowest bulk density, which correlated well with the observed porous surface morphology (Fig. 1B). Low bulk density value can be a qualitative indicator of the formation of hollow microspheres and/or lack of optimum packing of irregular (non-spherical) microparticles. Bulk density values

can also be correlated with specific surface area values; formulations with lower bulk density having higher specific surface areas-internal plus external. In this study, 8.6 kDa microspheres with the lowest bulk density had the highest total specific surface area. Formation of porous or hollow microspheres with high specific surface area in general translates into a faster drug release [18]. No major differences in bulk density were observed among 28.3 kDa, and polymer combination microspheres which were predominantly from the 28.3 kDa polymer. The specific surface areas with formulations C–E were higher as compared to that of formulation A, however, not as high as that was seen with 8.3 kDa polymer formulation B

Figs. 2 and 3 show the in vitro cumulative release of peptide. As expected, drug release from 8.6 kDa microspheres was very rapid, with approximately 55% of encapsulated drug being released within 24 h. This high initial release can be attributed to the more rapid hydration of lower molecular weight polymer as well as higher specific surface area. The high initial release was followed by a slower uniform release until exhaustion after 30 days. In contrast, drug release from 28.3 kDa microspheres was very slow and gradual up to 14 days, at which time the polymer apparently started dissolving and subsequently, polymer erosion controlled the drug release. A clear modi-
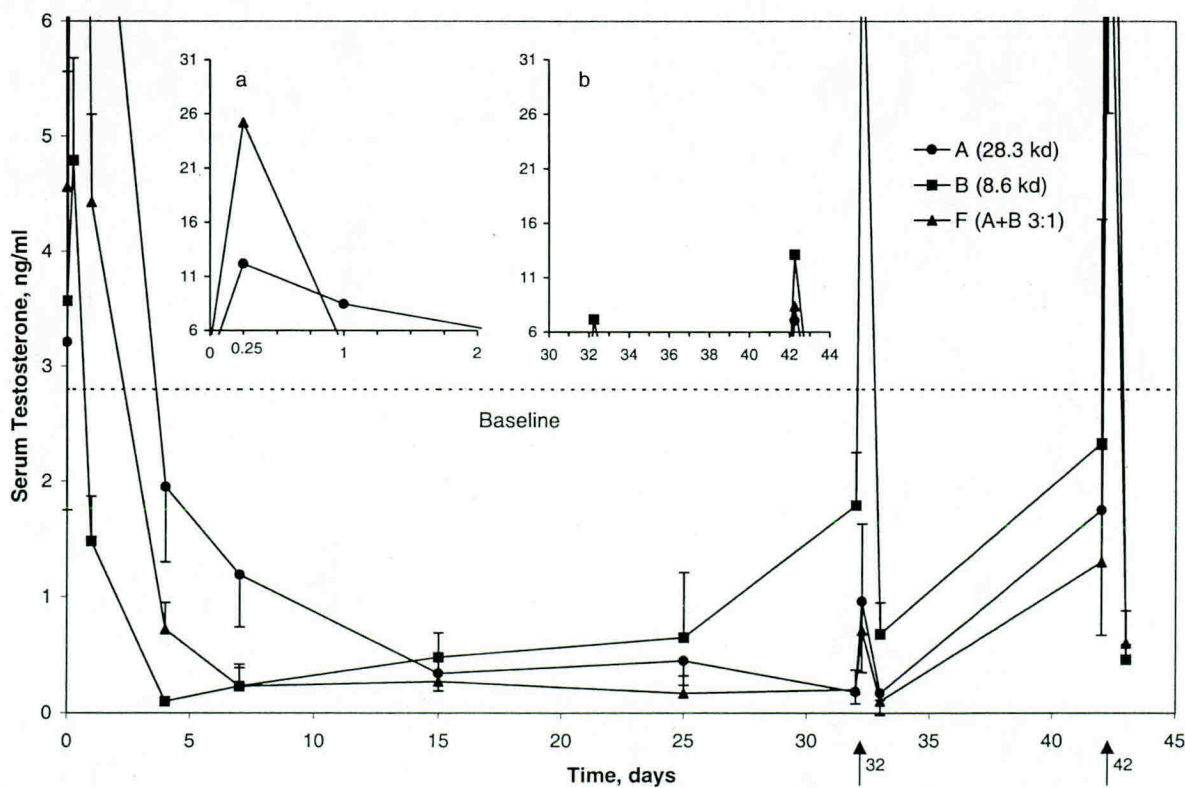

Fig. 4. In vivo testosterone suppression (SEM) with individual polymers, A and B, and microsphere blend, F, formulations. The inserts a and b show the data in the extended range above 6 ng/ml. Mean zero time values for the groups are shown on the y-axis while a mean baseline value from 180 rats is shown by the dotted line. ↑ indicates the challenge.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.

fastcase®
Smarter legal research.